CENTRAL AIRWAY ADMINISTRATION FOR SYSTEMIC DELIVERY OF THERAPEUTICS

Field of the Invention

The present invention relates to methods and products for the transepithelial delivery of therapeutics. In particular, the invention relates to methods and compositions for the systemic delivery of therapeutics conjugated to a neonatal Fc receptor (FcRn) binding partner by their administration to central airways of the lung. The methods and compositions are useful for any indication for which the therapeutic is itself useful in the treatment or prevention of a disease, disorder, or other condition of a subject.

Background of the Invention

Transport of macromolecules across an epithelial barrier may occur by receptor-nonspecific or receptor-specific mechanisms. Receptor-nonspecific mechanisms are represented by paracellular sieving events, the efficiency of which are inversely related to the molecular weight of the transported molecule. Transport of macromolecules such as immunoglobulin G (IgG) via this paracellular pathway is highly inefficient due to the large molecular mass of IgG (ca. 150 kDa). Receptor-nonspecific transport may include transcytosis in the fluid phase. This is much less efficient than receptor-mediated transport, because most macromolecules in the fluid phase are sorted to lysosomes for degradation. In contrast, receptor-specific mechanisms which may provide highly efficient transport of molecules otherwise effectively excluded by paracellular sieving. Such receptor-mediated mechanisms may be understood teleologically as effective scavenger mechanisms for anabolically expensive macromolecules such as albumin, transferrin, and immunoglobulin. These and other macromolecules would otherwise be lost at epithelial barriers through their diffusion down an infinite concentration gradient from inside to outside the body. Receptor-specific mechanisms for transport of macromolecules across epithelia exist for only a few macromolecules.

The surfaces defining the boundary between the inside of the body and the external world are provided by specialized tissue called epithelium. In its simplest form, epithelium is a single layer of cells of a single type, forming a covering of an external or "internal" surface. Epithelial tissues arise from endoderm and ectoderm and thus include skin, epithelium of the

cornea (eye), as well as the "internal" lining surfaces of the gastrointestinal tract, genitourinary tract, and respiratory system. These "internal" lining surfaces communicate with the external world, and thus they form a boundary between the inside of the body and the external world. While these various epithelia have specialized structural features or appendages that distinguish them, they also share much in common.

Two features common among various epithelia are the combination of large surface area on a gross level and close apposition with tight junctions on a cellular level. These two features present potential advantages and disadvantages, respectively, for the use of epithelium as a site for systemic, non-invasive delivery of therapeutics. For example, the surface area of the lung epithelium in human adults is believed to be 140 m^2. This enormous surface therefore potentially presents a highly attractive site of administration for systemic delivery of therapeutic agents, provided, of course, the therapeutic agent can be delivered to the epithelium and then transported across the epithelium.

Yet a third feature characteristic of various epithelia, and of particular importance to the present invention, is the receptor-specific mechanism for transport across an epithelial barrier provided by FcRn (neonatal Fc receptor). This receptor was first identified in neonatal rat and mouse intestinal epithelia and shown to mediate transport of maternal IgG from milk to the blood-stream of the suckling rat or mouse. IgG transferred to the neonate by this mechanism is critical for immunologic defense of the newborn. Expression of FcRn in rat and mouse intestinal epithelia was reported to cease following the neonatal period. In humans, humoral immunity does not depend on neonatal intestinal IgG transport. Rather, it was believed that a receptor of the placental tissue was responsible for IgG transport. The receptor responsible for this transport had been sought for many years. Several IgG-binding proteins had been isolated from placenta. FcγRII was detected in placental endothelium and FcγRIII in syncytiotrophoblasts. Both of these receptors, however, showed a relatively low affinity for monomeric IgG. In 1994, Simister and colleagues reported the isolation from human placenta of a cDNA encoding a human homolog of the rat and mouse Fc receptor for IgG. Story CM et al. (1994) *J Exp Med* 180:2377-81. The complete nucleotide and deduced amino acid sequences were reported and are available as GenBank Accession Nos. U12255 and AAA58958, respectively.

Unlike the rodent intestinal FcRn, the human FcRn was unexpectedly discovered to be expressed in adult epithelial tissues. U.S. Patent Nos. 6,030,613 and 6,086,875. Specifically,

human FcRn was found to be expressed on lung epithelial tissue, as well as on intestinal epithelial tissue (Israel EJ et al. (1997) *Immunology* 92:69-74), renal proximal tubular epithelial cells (Kobayashi N et al. (2002) *Am J Physiol Renal Physiol* 282:F358-65), and other mucosal epithelial surfaces including nasal epithlium, vaginal surfaces, and biliary tree surfaces.

U.S. Patent No. 6,030,613 discloses methods and compositions for the delivery of therapeutics conjugated to an FcRn binding partner to intestinal epithelium, mucosal epithelium, and epithelium of the lung.

U.S. Patent No. 6,086,875 discloses methods and compositions for stimulating an immune response to an antigen by the delivery of an antigen conjugated to an FcRn binding partner to an FcRn-expressing epithelium, including epithelium of the lung.

It is widely believed that administration of a therapeutic to lung epithelium for systemic delivery of the therapeutic requires delivery to the deep lung, i.e., to periphery of the lung, because that is how to access the greatest amount of surface area available. Yu J et al. (1997) *Crit Rev Therapeutic Drug Carrier Systems* 14:395-453. In addition, the epithelium lining the deepest reaches of the lungs, the alveoli, is a monolayer of extremely thin cells. In contrast, the epithelium of more proximal airways of the lungs are considerably thicker, and they are equipped with cilia to facilitate clearance of materials that could otherwise accumulate in the more distal airways and alveoli and thereby interfere with gas exchange. Aerosol delivery systems and methods therefore have been developed with the goal of maximizing drug delivery to the deep lung. This typically requires a combination of factors related both to the aerosol generator, e.g., metered dose inhaler (MDI) device, and special inhalation techniques to be employed by the patient in using the aerosol generator. For example, a typical MDI may be designed to generate the smallest possible droplets or particles, and it may be fitted for use with a spacer device or attachment to trap and remove larger, lower-velocity particles from the aerosol. The user may typically have to coordinate discharge of the MDI with initiation of inspiration, rate and depth of inspiration, breath-holding, and the like, all in order to increase the likelihood of effective delivery of the active agent to the deepest reaches of the lungs. Needless to say, patient compliance and therapeutic efficacy are frequently compromised by these technical requirements.

Summary of the Invention

The present invention relates in part to the surprising discovery by the inventors that the expression of FcRn on pulmonary epithelium is more extensive in central airways than in peripheral airways. This density distribution of FcRn in pulmonary epithelium actually favors aerosol admininstration of a therapeutic agent to central airways, rather than to deep lung, when the therapeutic agent is administered as a conjugate of the therapeutic agent and an FcRn binding partner. It has been discovered according to the present invention that preferential administration of aerosolized FcRn binding partner conjugate to central airways permits highly efficient FcRn-mediated transcytosis of the conjugate across the respiratory epithlium and systemic delivery of the therapeutic agent. Unlike other methods and compositions for systemic delivery via pulmonary administration, the invention advantageously requires no special breathing techniques to effect optimal systemic delivery. The technical obstacles presented by the need for deep lung delivery are thereby averted, and the invention provides effective strategies useful for noninvasive, systemic delivery of a therapeutic agent to a subject through its aerosol administration to central airways of the lung as a conjugate with an FcRn binding partner.

The invention is useful wherever it is desirable to administer a particular therapeutic agent to a subject for the treatment or prevention of a condition of the subject that is treatable with the therapeutic agent. The invention can be particularly useful whenever repeated or chronic administration of a therapeutic agent is called for, compliance with a special breathing technique is difficult to achieve, as well as whenever invasive administration is preferably avoided.

According to one aspect of the invention, a method for systemic delivery of a therapeutic agent is provided. The method involves administering an effective amount of an aerosol of a conjugate of a therapeutic agent and an FcRn binding partner to lung such that a central lung zone/peripheral lung zone deposition ratio (C/P ratio) is at least 0.7. As explained further below, the C/P ratio is selected such that the conjugate is preferentially delivered to central airways.

The C/P ratio in a preferred embodiment according to this aspect of the invention is at least 1.0. In a more preferred embodiment the C/P ratio is at least 1.5. In a most preferred embodiment the C/P ratio is at least 2.0.

According to another aspect of the invention, a method is provided for systemic delivery of a therapeutic agent. The method involves administering an effective amount of an

...f a conjugate of a therapeutic agent and an FcRn binding partner to lung, wherein particles in the aerosol have a mass median aerodynamic diameter (MMAD) of at least 3 micrometers (μm).

According to yet another aspect, the invention provides an aerosol of a conjugate of a therapeutic agent and an FcRn binding partner, wherein particles in the aerosol have a MMAD of at least 3 μm.

According to still another aspect, the invention provides an aerosol delivery system. The aerosol delivery system according to this aspect includes a container, an aerosol generator connected to the container, and a conjugate of a therapeutic agent and an FcRn binding partner disposed within the container, wherein the aerosol generator is constructed and arranged to generate an aerosol of the conjugate having particles with a MMAD of at least 3 μm.

In one embodiment, this aspect provides a method of manufacturing the aerosol delivery system. The method involves the steps of providing the container, providing the aerosol generator connected to the container, and placing an effective amount of the conjugate in the container.

In some embodiments according to this aspect of the invention, the aerosol generator comprises a vibrational element in fluid connection with a solution containing the conjugate.

In some embodiments, the vibrational element comprises a member having (a) a front surface; (b) a back surface in fluid connection with the solution; and (c) a plurality of apertures traversing the member. In preferred embodiments, the apertures at the front surface are at least 3 μm in diameter. Preferably, the apertures are tapered so that they narrow from the back surface to the front surface.

In some embodiments according to this aspect of the invention, the aerosol generator is a nebulizer. In some embodiments, the nebulizer is a jet nebulizer.

In some embodiments according to this aspect of the invention, the aerosol generator is a mechanical pump.

In some embodiments according to this aspect of the invention, the container is a pressurized container.

According to still another aspect, the invention provides an aerosol delivery system. The aerosol delivery system according to this aspect includes a container, an aerosol generator

connected to the container, and a conjugate of a therapeutic agent and an FcRn binding partner disposed within the container, wherein the aerosol generator includes a means for generating an aerosol of the conjugate having particles with a MMAD of at least 3 μm.

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In each of the foregoing aspects of the invention, in some embodiments the MMAD of the particles is between 3 μm and about 8 μm. In some embodiments the MMAD of the particles is greater than 4 μm. In preferred embodiments a majority of the particles are non-respirable, i.e., they have a MMAD of at least 4.8 μm. Non-respirable particles are believed not to enter the alveolar space in the deep lung.

In each of the foregoing aspects of the invention, in some embodiments the FcRn binding partner contains a ligand for FcRn which mimics that portion of the Fc domain of IgG which binds the FcRn (i.e., an Fc, an Fc domain, Fc fragment, Fc fragment homolog). In preferred embodiments, the FcRn binding partner is non-specific IgG or an FcRn-binding fragment of IgG. Most typically the FcRn binding partner corresponds to the Fc fragment of IgG.

In each of the foregoing aspects of the invention, in some embodiments the therapeutic agent and the FcRn binding partner are coupled by a covalent bond.

In each of the foregoing aspects of the invention, in some embodiments the therapeutic agent and the FcRn binding partner are coupled by a linker. Preferably the linker is a peptide linker. In some embodiments the linker comprises at least part of a substrate for an enzyme that specifically cleaves the substrate.

In each of the foregoing aspects of the invention, in some embodiments the therapeutic agent is a polypeptide. The conjugate in such embodiments is preferably an isolated fusion protein. In certain such embodiments, the polypeptide therapeutic agent of the conjugate may be linked to the FcRn binding partner by a linker, provided the polypeptide therapeutic agent and the FcRn binding partner each retains at least some of its biological activity.

In each of the foregoing aspects of the invention, in some embodiments the therapeutic agent is a cytokine. In some embodiments the therapeutic agent is a cytokine receptor or a cytokine-binding fragment thereof.

In each of the foregoing aspects of the invention, in some embodiments the therapeutic agent is an antigen. The antigen may be characteristic of a pathogen, characteristic of an autoimmune disease, characteristic of an allergen, or characteristic of a tumor. In certain preferred embodiments the antigen is a tumor antigen.

In each of the foregoing aspects of the invention, in some embodiments the therapeutic agent is an oligonucleotide. In certain preferred embodiments the oligonucleotide is an antisense oligonucleotide.

In each of the foregoing aspects of the invention, in some embodiments the therapeutic agent is erythropoietin (EPO), growth hormone, interferon alpha (IFN-α), interferon beta (IFN-β), or follicle stimulating hormone (FSH). In each of the foregoing aspects of the invention, in some embodiments the therapeutic agent is Factor VIIa, Factor VIII, Factor IX, tumor necrosis factor-alpha (TNF-α), TNF-α receptor (for example, etanercept, ENBREL®; see U.S. Patent No. 5,605,690, PCT/US93/08666 (WO 94/06476), and PCT/US90/04001 (WO 91/03553)), lymphocyte function antigen-3 (LFA-3), ciliary neurotrophic factor (CNTF). In certain preferred embodiments the therapeutic agent is EPO. In other preferred embodiments the therapeutic agent is growth hormone. In other preferred embodiments the therapeutic agent is IFN-α. In yet other preferred embodiments the

therapeutic agent is IFN-β. In still other preferred embodiments the therapeutic agent is FSH. In a preferred embodiment the therapeutic agent is Factor VIII. In another preferred embodiment the therapeutic agent is Factor IX. In yet another preferred embodiment the therapeutic agent is TNF-α. In a preferred embodiment the therapeutic agent is a TNF receptor. In yet another preferred embodiment the therapeutic agent is LFA-3. In a further preferred embodiment the therapeutic agent is CNTF. In each and every one of these and like embodiments, the therapeutic agent is a biologically active polypeptide, whether whole or a portion thereof. For example, a therapeutic agent that is a TNF receptor (TNFR) includes whole TNFR as well as a TNF-binding TNF receptor polypeptide, e.g., an extracellular domain of TNFR.

In each of the foregoing aspects of the invention, in certain preferred embodiments the conjugate is substantially in its native, non-denatured form. In some embodiments at least 60 percent of the conjugate is in its native, non-denatured form. In more preferred embodiments at least 70 percent of the conjugate is in its native, non-denatured form. In even more preferred embodiments at least 80 percent of the conjugate is in its native, non-denatured form. In highly preferred embodiments at least 90 percent of the conjugate is in its native, non-denatured form. In even more highly preferred embodiments at least 95 percent of the conjugate is in its native, non-denatured form. In most highly preferred embodiments at least 98 percent of the conjugate is in its native, non-denatured form.

These and other aspects of the invention are described in greater detail below.

Brief Description of the Figures

Figure 1 presents nucleotide (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of human IgG1 Fc fragment including the hinge, C_H2, and C_H3 domains. Numbers beneath the amino acid sequence correspond to the amino acid designations using the EU numbering convention.

Figure 2 presents cDNA open reading frame nucleotide (Panel A; SEQ ID NO:3) and deduced amino acid (Panel B; SEQ ID NO:4) sequences of wildtype human EPO. The signal peptide in SEQ ID NO:4 is underlined.

Figure 3 presents a plasmid map for expression plasmid pED.dC.XFc (Panel A) and the nucleotide (SEQ ID NO:5) and amino acid (SEQ ID NO:6) sequences of the K^b signal peptide/Fcγ1 insert (Panel B). The K^b signal peptide and the Fcγ1 regions are indicated by a

tilde (~) above the sequence. The *Eco*RI, *Pst*I and *Xba*I restriction enzyme sites are underlined.

Figure 4 presents a plasmid map for expression plasmid pED.dC.EpoFc (Panel A) and the nucleotide (SEQ ID NO:7) and amino acid (SEQ ID NO:8) sequences of the K^b signal peptide/EPO/Fcγ1 insert (Panel B). The K^b signal peptide, mature EPO, and Fcγ1 regions are indicated by a tilde (~) above the sequence. The *Eco*RI, *Sbf*I and *Xba*I restriction enzyme sites are underlined.

Figure 5 presents a plasmid map for expression plasmid pED.dC.natEpoFc (Panel A) and the nucleotide (SEQ ID NO:9) and amino acid (SEQ ID NO:10) sequences of the nativeEPO/Fcγ1 insert (Panel B). The mature EPO, including the native EPO signal peptide, and Fcγ1 regions are indicated by a tilde (~) above the sequence. The *Eco*RI, *Pst*I and *Xba*I restriction enzyme sites are underlined.

Figure 6 is a pair of graphs depicting in vivo response to EPO-Fc administered as an aerosol to central airways of cynomolgus monkeys. Panel A shows maximum reticulocyte response for each of nine animals. Aerosolized EPO-Fc was administered to spontaneously breathing animals using a nebulizer. Panel B shows the maximum serum concentration of EPO-Fc (native Fc fragment) and mutant EPO-Fc (Fc fragment having mutations of three amino acids critical for FcRn binding) following inhalation by shallow or deep breathing.

Figure 7 is a graph depicting the maximum serum concentration of EPO-Fc in cynomolgus monkeys following aerosol administration at 20% vital capacity (20% VC, shallow breathing) and 75% vital capacity (75% VC, deep breathing).

Figure 8 is a graph depicting serum concentration over time of EPO-Fc in cynomolgus monkeys following aerosol administration at 20% vital capacity at doses of 30 μg/kg (circles) and 10 μg/kg (triangles). Each curve represents data from a single animal.

Figure 9 is a graph depicting serum concentration over time of IFN-α-Fc or IFN-α alone in cynomolgus monkeys following aerosol administration of IFN-α-Fc or INTRON® A using shallow breathing at doses of 20 μg/kg. Each curve represents data from a single animal.

Figure 10 is a graph depicting serum concentration over time of IFN-α-Fc in cynomolgus monkeys following aerosol administration of IFN-α-Fc using shallow breathing at doses of 2 μg/kg. Each curve represents data from a single animal.

Figure 11 is a pair of graphs depicting oligoadenylate synthetase (OAS) activity (panel A) and neopterin concentration (panel B), two common measures of IFN-α bioactivity, following aerosol administration of IFN-α-Fc using shallow breathing at doses of 20 μg/kg. Each curve represents data from a single animal.

Figure 12 is a graph depicting serum concentration over time of ENBREL® (human TNFR-Fc) in cynomolgus monkeys following aerosol administration of IFN-α-Fc using shallow breathing at estimated deposited doses of 0.3-0.5 mg/kg. Each curve represents data from a single animal.

Detailed Description of the Invention

The invention is useful whenever it is desirable to deliver a therapeutic agent across lung epithelium to effect systemic delivery of the therapeutic agent. This is accomplished by administering to central airways a conjugate of a therapeutic agent with an FcRn binding partner, where the central airways are by nature peculiarly suited for FcRn receptor-mediated transcellular transport of FcRn binding partners. Advantageously, the invention may be used in the systemic delivery of therapeutics of nearly any size, including those having very large molecular weight. The invention thus may be used for the pulmonary administration of macromolecules, peptides, oligonucleotides, small molecules, drugs, and diagnostic agents for systemic delivery.

The invention in one aspect provides a method for delivery of a therapeutic agent, wherein the method involves administering an effective amount of an aerosol of a conjugate of a therapeutic agent and an FcRn binding partner to lung such that a C/P ratio is at least 0.7.

A "therapeutic agent" as used herein refers to a compound useful to treat or prevent a disease, disorder, or condition of a subject. As used herein, the term "to treat" means to ameliorate the signs or symptoms of, or to stop the progression of, a disease, disorder, or condition of a subject. Signs, symptoms, and progression of a particular disease, disorder, or condition of a subject can be assessed using any applicable clinical or laboratory measure recognized by those of skill in the art, e.g., as described in Harrison's Principles of Internal Medicine, 14th Ed., Fauci AS et al., eds., McGraw-Hill, Ne : York, 1998. As used herein, the term "subject" means a mammal and preferably a human. For treating or preventing a particular disease, disorder, or condition, those of skill in the art will recognize a suitable therapeutic agent for that purpose.

The FcRn binding partner conjugates of the present invention may be utilized for the systemic delivery of a wide variety of therapeutic agents, including but not limited to, antigens, including tumor antigens; chemotherapy agents for the treatment of cancer; cytokines; growth factors; nucleic acid molecules and oligonucleotides, including DNA and RNA; hormones; fertility drugs; calcitonin, calcitriol and other bioactive steroids; antibiotics, including antibacterial agents, antiviral agents, antifungal agents, and antiparasitic agents; cell proliferation-stimulating agents; lipids; proteins and polypeptides; glycoproteins; carbohydrates; and any combination thereof. Specific examples of therapeutic agents are presented elsewhere herein. The FcRn binding partners of the present invention may further be utilized for the targeted delivery of a delivery vehicle, such as microparticles and liposomes.

An "aerosol" as used herein refers to a suspension of liquid or solid in the form of fine particles dispersed in a gas. As used herein, the term "particle" thus refers to liquids, e.g., droplets, and solids, e.g., powders. Pharmaceutical aerosols for the delivery of conjugates of the invention to the lungs are preferably inhaled via the mouth, and not via the nose. Alternatively, pharmaceutical aerosols for the delivery of conjugates of the invention to the lungs are preferably introduced through direct delivery to a central airway, for example via an endotracheal tube or tracheostomy.

As described in further detail below, a "conjugate" as used herein refers to two or more entities bound to one another by any physicochemical means, including, but not limited to, covalent interaction, hydrophobic interaction, hydrogen bond interaction, or ionic interaction. It is important to note that the bond between the FcRn binding partner and the therapeutic agent must be of such a nature and location that it does not destroy the ability of the FcRn binding partner to bind to the FcRn. Such bonds are well known to those of ordinary skill in the art, and examples are provided in greater detail below. The conjugate further may be formed as a fusion protein, also discussed in greater detail below.

The conjugate may include an intermediate or linker entity between the therapeutic agent and the FcRn binding partner, such that the therapeutic agent and the FcRn binding partner are bound to one another indirectly. In some embodiments the linker is subject to spontaneous cleavage. In some embodiments the linker is subject to assisted cleavage by an agent such as an enzyme or chemical. For example, protease-cleavable peptide linkers are well known in the art and include, without limitation, trypsin-sensitive sequence; plasmin-

sensitive sequence; FLAG peptide; chymosin-sensitive sequence of bovine κ-casein A (Walsh MK et al. (1996) *J Biotechnol* 45:235-41); cathepsin B cleavable linker (Walker MA et al. (2002) *Bioorg Med Chem Lett* 12:217-9); thermolysin-sensitive poly(ethylene glycol) (PEG)-L-alanyl-L-valine (Ala-Val) (Suzawa T et al. (2000) *J Control Release* 69:27-41); enterokinase-cleavable linker (McKee C et al. (1998) *Nat Biotechnol* 16:647-51). Protease-cleavable peptide linkers may be designed for use and used in association with other major classes of proteases, e.g., matrix metalloproteinases and secretases (sheddases). Birkedal-Hansen H et al. (1993) *Crit Rev Oral Biol Med* 4:197-250; Hooper NM et al. (1997) *Biochem J* 321(Pt 2):265-79. In other embodiments the linker may be resistant to spontaneous, proteolytic, or chemical cleavage. An example of this type of linker is arginine-lysine-free linker (resistant to trypsin). Additional examples of linkers include, without limitation, polyglycine, $(Gly)_n$; polyalanine, $(Ala)_n$; poly(Gly-Ala), $(Gly_m\text{-}Ala)_n$; poly (Gly-Ser), (e.g., $Gly_m\text{-}Ser)_n$, and combinations thereof, where m and n are each independently an integer between 1 and 6. See also Robinson CR et al. (1998) *Proc Natl Acad Sci USA* 95:5929-34.

An "FcRn binding partner" as used herein refers to any entity that can be specifically bound by the FcRn with consequent active transport by the FcRn of the FcRn binding partner. FcRn binding partners of the present invention thus encompass, for example, whole IgG, the Fc fragment of IgG, other fragments of IgG that include the complete binding region for the FcRn, and other molecules that mimic FcRn-binding portions of Fc and bind to FcRn. In certain embodiments the FcRn binding partner excludes FcRn-specific whole antibodies which specifically bind FcRn through antigen-specific antigen-antibody interaction. It is to be understood in this context that antigen-specific antigen-antibody interaction means antigen binding specified by at least one complementarity determining region (CDR) within a hypervariable region of an antibody, e.g., a CDR within Fab, F(ab'), $F(ab')_2$, and Fv fragments. Likewise, in certain embodiments the FcRn binding partner excludes FcRn-specific fragments, and analogs of FcRn-specific fragments, of whole antibodies which specifically bind FcRn through antigen-specific antigen-antibody interaction. Some such embodiments thus exclude FcRn-specific Fv fragments, single chain Fv (scFv) fragments, and the like. Other such embodiments exclude FcRn-specific Fab fragments, F(ab') fragments, $F(ab')_2$ fragments, and the like.

A "C/P ratio" is a measure of relative distribution of deposition of aerosolized particles to central airways of the lung in comparison to deposition to the periphery of the

lung. "Central airways" refers to conducting and transitional airways, distal to the larynx, which have little to no role in gas exchange. In humans central airways include the trachea, main bronchi, lobar bronchi, segmental bronchi, small bronchi, bronchioles, terminal bronchioles, and respiratory bronchioles. The central airways thus account for the first 16-19 generations of airway branching in the lung, where the trachea is generation zero (0) and the alveolar sac is generation 23. Wiebel ER (1963) Morphometry of the Human Lung, Berlin:Springer-Verlag, pp. 1-151. The terms "periphery of the lung" and, equivalently, "deep lung" refer to airways of the lung distal to the central airways. The central airways are responsible for the bulk movement of air, as opposed to the periphery of the lung, which is primarily responsible for gas exchange between air and blood. In aggregate, the central airways account for only about ten percent of the entire respiratory epithelial surface area of the lungs. Qiu Y et al. (1997) In: Inhalation Delivery of Therapeutic Peptides and Proteins, Adjei AL and Gupta PK, eds., Lung Biology in Health and Disease, Vol. 107, Marcel Dekker: New York, pp. 89-131.

Notably, epithelial cell types vary beween the central and peripheral regions of the lung. Central airways are lined by ciliated columnar epithelial cells and cuboidal epithelial cells, whereas the respiratory zone is lined by cuboidal epithelial cells and, more distally, alveolar epithelial cells. Whereas the distance across alveolar epithelium is very small, i.e., 0.1 - 0.2 μm, the distance across columnar and cuboidal epithelial cells is many times greater, e.g., 30 - 40 μm for columnar epithelium.

Those of skill in the art typically refer to the P/C ratio or, equivalently, the penetration index, as a measure of effective administration of agents to the deep lung. As the term suggests, the P/C ratio is a measure of relative distribution of deposition of aerosolized particles to the periphery of the lung in comparison to deposition to the central airways of the lung; it is thus the arithmetic inverse of the C/P ratio. The P/C ratio varies directly with the result that has until now typically been sought in order to achieve systemic delivery of the inhaled agent, i.e., preferential administration to the deep lung. Typical P/C ratios sought for conventional applications are in the range of about 1.35 to 2.2 and higher.

Unlike these more typical applications, which call for maximizing administration to the periphery of the lung and thus a high P/C ratio, in the instant invention it is desirable to focus administration to the central airways of the lung. Thus in the instant invention it is desirable to achieve a relatively low P/C ratio, i.e., a high C/P ratio, in accordance with the

surprising discovery that administration to the central airways is preferred to administration to the periphery of the lung. Accordingly, the C/P ratio varies directly with the result that is sought in the instant invention, i.e., preferential administration to the central airways of the lung. Accordingly, preferred embodiments include those for which the C/P ratio is at least 0.7 - 0.9. These embodiments specifically include those having C/P ratios of at least 0.7, 0.8, and 0.9. More preferred embodiments include those for which the C/P ratio is at least 1.0 - 1.4. These embodiments specifically include those having C/P ratios of at least 1.0, 1.1, 1.2, 1.3, and 1.4. Even more preferred embodiments include those for which the C/P ratio is at least 1.5 - 1.9. These embodiments specifically include those having C/P ratios of at least 1.5, 1.6, 1.7, 1.8, and 1.9. Most preferred embodiments include those for which the C/P ratio is at least: 2.0 - 3.0. These embodiments specifically include those having C/P ratios of at least 2.0, 2.1, 2.2, 2.3. 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, and 3.0. There is no theoretical upper limit of the C/P ratio. Thus most preferred embodiments include those having C/P ratios greater than 3.0.

Determination of the C/P ratio can be accomplished by any suitable method, but typically such determination involves planar imaging gamma scintigraphy, three-dimensional single-photon emission computed tomography (SPECT), or positron emission tomography (PET). Newman SP et al. (1998) *Respiratory Drug Delivery* VI:9-15; Fleming JS et al. (2000) *J Aerosol Med* 13:187-98. In a typical determination of the P/C ratio, an appropriate gamma ray emitting radionuclide, e.g., ^{99m}Tc, ^{113m}In, ^{131}I, or ^{81m}Kr, is added to the drug formulation. After aerosol administration to a subject, data is acquired with a gamma camera and analysed by dividing the resulting lung images into two (central and peripheral) or three (central, intermediate, and peripheral) imaging regions. Newman SP et al., supra; Agnew JE et al. (1986) *Thorax* 41:524-30. Depending on the selected imaging method, the central imaging region or the central and intermediate imaging regions together are representative of central airways. The peripheral imaging region is representative of the periphery of the lung. Taking attenuation and decay into account, counts from the peripheral imaging region are divided by counts from the central imaging region (or, where appropriate, by combined counts from the central and intermediate imaging regions). Determination of the C/P ratio follows the method just outlined, but the ratio is calculated as counts from the central imaging region (or, where appropriate, combined counts from the central and intermediate imaging regions), divided by counts from the peripheral zone.

A number of factors contribute to the site of particle deposition within the lung, including the mechanics of breathing. Generally, the faster, shallower, and shorter the duration of inspiration, the more favorable for deposition in the central airways. Conversely, the slower, deeper, and longer the duration of inspiration, the more favorable for deposition in the periphery of the lung. Thus for example normal (i.e., tidal) breathing favors deposition in the central airways, whereas deep, supranormal inspiration and breath-holding favor deposition in the deep lung. Put another way, low flow, low pressure respiration favors deposition in the central airways, and conversely high flow, high pressure respiration favors deposition in the deep lung. Accordingly, in the setting of respiration on a mechanical ventilator, flow and pressure parameters controlled by the mechanical ventilator can be set to favor either central or peripheral deposition in the lungs. Such parameters for mechanically controlled or assisted breathing are selected on the basis of a number of clinical factors well known in the art, including body weight, underlying pulmonary or other disease, fraction of inspired oxygen (FiO_2), fluid volume status, lung compliance, etc., as well as the effective gas exchange as reflected by, e.g., blood pH, partial pressure of oxygen in the blood, and partial pressure of carbon dioxide in the blood.

Achievement of a C/P ratio of at least 0.7 is therefore favored by use of a normal or tidal breathing pattern as part of the preferred method of administration. This may be accomplished, for example, by inhaling an aerosol over the course of a number of breaths during tidal breathing. In the setting of respiration on a mechanical ventilator, achievement of a C/P ratio of at least 0.7 is therefore favored by low flow, low pressure assisted ventilation as part of the preferred method of administration.

Another factor affecting the the site and extent of particle deposition within the airways relates to physicochemical characteristics of the particles. Important physicochemical characteristics of the particles include their aerodynamic diameter, mass density, velocity, and electrical charge. Some of these factors are considered in the following aspect of the invention.

According to another aspect of the invention, a method is provided for systemic delivery of a therapeutic agent. The method according to this aspect involves administering an effective amount of an aerosol of a conjugate of a therapeutic agent and an FcRn binding partner to lung, wherein particles in the aerosol have a mass median aerodynamic diameter (MMAD) of at least 3 μm. According to yet another aspect, the invention provides an aerosol

of a conjugate of a therapeutic agent and an FcRn binding partner, wherein particles in the aerosol have a MMAD of at least 3 μm. Particle size and distribution are believed to be important parameters influencing aerosol deposition. Aerosol particles generally range in shape and size. The individual particle sizes of an aerosol may be characterized microscopically and an average primary particle size value can then be estimated, which describes the central tendency of the entire size distribution. It is convenient to express the particle size of irregularly shaped particles by an equivalent spherical dimension. The aerodynamic diameter (D_{ae}) is defined as the diameter of a unit density sphere having the same settling velocity (generally in air) as the particle being studied. This dimension encompasses the particle's shape, density and physical size. A population of particles can be defined in terms of the mass carried in each particle size range. This distribution can be divided into two equal halves at the mass median aerodynamic diameter (MMAD). The distribution around the MMAD may be expressed in terms of the geometric standard deviation (GSD). These parameters can be used if it is assumed that aerosol particle size distributions are log-normal.

Because particle size may not be homogeneous, in various embodiments the particles having a D_{ae} of at least 3 μm may constitute at least 50 percent, at least 60 percent, at least 70 percent, preferably at least 75 percent, more preferably at least 80 percent, even more preferably at least 85 percent, even more preferably at least 90 percent, and most preferably at least 95 percent of the particles in the aerosol.

The mechanisms of deposition of aerosol particles within airways include inertial impaction, interception, sedimentation, and diffusion. Inertial impaction occurs when large (high-mobility) particles or droplets travel in their initial direction of motion and do not follow the velocity streamlines as the direction of motion of the air passes around obstructions. These large particles travel to the obstruction and are deposited. Inertial impaction occurs throughout the tracheobronchial tree but particularly in the largest airways, where flow velocity and particle size are much larger. Interception is relevant in nasal deposition and in small airways. Particles will be intercepted when they enter an airstream moving in a direction of flow located less than the particles' diameter from the airway wall. Sedimentation takes place under the force of gravity and affects particles that are relatively large and are located in smaller airways of the alveolar region. Diffusion is responsible for the deposition of small, submicrometer particles. Particles move randomly under the

influence of impact by gas molecules until they travel to the wall of the airway.

Specialized aerosol generators are known to be capable of creating "monodisperse" aerosols, i.e., aerosols with particles having a GSD of less than 1.2 μm. Fuchs NA et al. (1966) In: Davies CN, ed., Aerosol Science, London: Academic Press, pp. 1-30. The vibrating orifice monodisperse aerosol generator (VOAG) is an example of one type of monodisperse aerosol generator, and it is frequently employed to prepare calibration standards. Berglund RN et al. (1973) *Environ Sci Technol* 7:147. This generator can achieve GSDs approaching 1.05 when concentrate is fed through the orifice plate having orifice diameters that range in size from 5 to 50 μm. Additional types of monodisperse aerosol generators include spinning disk and spinning top aerosol generators. These too are frequently employed to prepare calibration standards.

Particle size, i.e., MMAD and GSD, can be measured using any suitable technique. Techniques widely employed include single- and multi-stage inertial impaction, virtual impaction, laser particle sizing, optical microscopy, and scanning electron microscopy. For a review, see Lalor CB et al. (1997) In: Inhalation Delivery of Therapeutic Peptides and Proteins, Adjei AL and Gupta PK, eds., New York: Marcel Dekker, pp 235-276.

Particle sizes in the range 2 μm to 10 μm are widely considered to be optimal for the delivery of therapeutic agents to the tracheobronchial and pulmonary regions. Heyder J et al. (1986) *J Aerosol Sci* 17:811-25. Maximal alveolar deposition has been shown to occur when particles have diameters between 1.5 μm and 2.5 μm and between 2.5 μm and 4 μm, with and without breath-holding techniques, respectively. Byron PR (1986) *J Pharm Sci* 75:433-38. As particle sizes increase beyond about 3 μm, deposition decreases in the alveoli and increases in the central airways. Beyond about 10 μm, deposition occurs predominantly in the larynx and upper airways.

As mentioned previously, particles having a MMAD of at least 4.8 μm are non-respirable, i.e., they are believed not to enter the alveolar space in the deep lung. This explains why, prior to now, it has generally been preferred to administer aerosols characterized by particles having a MMAD of less than 5 μm. By contrast, in certain preferred embodiments of the instant invention, a majority of the particles are non-respirable.

In yet another aspect the invention provides an aerosol delivery system. The aerosol delivery system according to this aspect includes a container, an aerosol generator connected

to the container, and a conjugate of a therapeutic agent and an FcRn binding partner disposed within the container, wherein the aerosol generator is constructed and arranged to generate an aerosol of the conjugate having particles with a MMAD of at least 3 μm.

In a particularly preferred embodiment the aerosol delivery system includes a vibrational element constructed and arranged to vibrate an aperture plate having a plurality of apertures of defined geometry, wherein one side or surface of the aperture plate is in fluid connection with a solution or suspension of the conjugate. See, e.g., U.S. Patent No. 5,758,637, U.S. Patent No. 5,938,117, U.S. Patent No. 6,014,970, U.S. Patent No. 6,085,740, and U.S. Patent No. 6,205,999, the entire contents of which are incorporated by reference. Activation of the vibrational element to vibrate the aperture plate causes liquid containing the conjugate in solution or suspension to be drawn through the plurality of apertures to create a low-velocity aerosol with a defined range of droplet (i.e., particle) sizes.

Examples of this type of aerosol generator are commercially available from Aerogen, Inc., Sunnyvale, California.

In another embodiment the aerosol delivery system includes a pressurized container containing the conjugate in solution or suspension. The pressurized container typically has an actuator connected to a metering valve so that activation of the actuator causes a predetermined amount of the conjugate in solution or suspension within the container to be dispensed from the container in the form of an aerosol. Pressurized containers of this type are well known in the art as propellant-driven metered-dose inhalers (pMDIs or simply MDIs). MDIs typically include an actuator, a metering valve, and a pressurized container that holds a micronized drug suspension or solution, liquefied propellant, and surfactant (e.g., oleic acid, sorbitan trioleate, lecithin). Historically these MDIs typically used chlorofluorocarbons (CFCs) as propellants, including trichlorofluoromethane, dichlorodifluoromethane, and dichlorotetrafluoromethane. Cosolvents such as ethanol may be present when the propellant alone is a relatively poor solvent. Newer propellants may include 1,1,1,2-tetrafluoroethane and 1,1,1,2,3,3,3-heptafluoropropane. Actuation of MDIs typically causes dose amounts of 50 μg-5 mg of active agent in volumes of 20-100 μL to be delivered at high velocity (30 m/sec) over 100-200 msec.

In other embodiments the aerosol delivery system includes an air-jet nebulizer or ultrasonic nebulizer in fluid connection with a reservoir containing the conjugate in solution or suspension. Nebulizers (air-jet or ultrasonic) are used primarily for acute care of

nonambulatory patients and in infants and children. Air-jet nebulizers for atomization are considered portable because of the availability of small compressed air pumps, but they are relatively large and inconvenient systems. Ultrasonic nebulizers have the advantage of being more portable because they generally do not require a source of compressed air. Nebulizers provide very small droplets and high mass output. Doses administered by nebulization are much larger than doses in MDIs and the liquid reservoir is limited in size, resulting in short, single-duration therapy.

To generate an aerosol from an air-jet nebulizer, compressed air is forced through an orifice over the open end of a capillary tube, creating a region of low pressure. The liquid formulation is drawn through the tube to mix with the air jet and form the droplets. Baffles within the nebulizer remove larger droplets. The droplet size in the airstream is influenced by the compressed air pressure. Mass median diameters normally range from 2 to 5 μm with air pressures of 20 to 30 psig. The various commercially available air-jet nebulizers do not perform equally. This will affect the clinical efficacy of nebulized aerosol, which depends on the droplet size, total output from the nebulizer, and patient determinants.

Ultrasonic nebulizers generate aerosols using high-frequency ultrasonic waves (i.e., 100 kHz and higher) focused in the liquid chamber by a ceramic piezoelectric crystal that mechanically vibrates upon stimulation. Dennis JH et al. (1992) *J Med Eng Tech* 16:63-68; O'Doherty MJ et al. (1992) *Am Rev Respir Dis* 146:383-88. In some instances, an impeller blows the particles out of the nebulizer or the aerosol is inhaled directly by the patient. The ultrasonic nebulizer is capable of greater output than the air-jet nebulizer and for this reason is used frequently in aerosol drug therapy. The droplets formed using ultrasonic nebulizers, which depend upon the frequency, are coarser (i.e., higher MMAD) than those delivered by air-jet nebulizers. The energy introduced into the liquid can result in an increase in temperature, which results in vaporization and variations in concentrations over time. This concentration variation over time is also encountered in jet nebulizers but is due to water loss through evaporation.

The choice between solution or suspension formulations in nebulizers is similar to that for the MDI. The formulation chosen will affect total mass output and particle size. Nebulizer formulations typically contain water with cosolvents (ethanol, glycerin, propylene glycol) and surfactants added to improve solubility and stability. Commonly an osmotic agent is also added to prevent bronchoconstriction from hypoosmotic or hyperosmotic

solutions. Witeck TJ et al. (1984) *Chest* 86:592-94; Desager KN et al. (1990) *Agents Actions* 31:225-28.

In yet other embodiments the aerosol delivery system includes a dry powder inhaler in fluid connection with a reservoir containing the conjugate in powder form. The dry powder inhaler device may eventually replace MDIs for some indications in response to the international control of chlorofluorocarbons in these latter products. Notably, this device can only deliver a fraction of its load in a respirable size range. Powder inhalers will usually disperse only about 10 to 20% of the contained drug into respirable particles. The typical dry powder inhaler device consists of two elements: the inhalation appliance to disperse unit doses of the powder formulation into the inspired airstream, and a reservoir of the powder formulation to dispense these doses. The reservoir typically can be of two different types. A bulk reservoir allows a precise quantity of powder to be dispensed upon individual dose delivery up to approximately 200 doses. A unit dose reservoir provides individual doses (e.g., provided in blister packaging or in gelatin capsule form) for inhalation as required. The hand-held device is designed to be manipulated to break open the capsule/blister package or to load bulk powder followed by dispersion from the patient's inspiration. Airflow will deaggregate and aerosolize the powder. In most cases, the patient's inspiratory airflow activates the device, provides the energy to disperse and deagglomerate the dry powder, and determines the amount of medicament that will reach the lungs.

Dry powder generators are subject to variability because of the physical and chemical properties of the powder. These inhalers are designed to meter doses ranging from 200 μg to 20 mg. The preparation of drug powder in these devices is very important. The powder in these inhalers requires efficient size reduction that is also needed for suspensions in MDIs. Micronized particles flow and are dispersed more unevenly than coarse particles. Therefore the micronized drug powder may be mixed with an inert carrier. This carrier is usually α-lactose monohydrate, because lactose comes in a variety of particle size ranges and is well characterized. Byron PR et al. (1990) *Pharm Res* 7(suppl):S81. The carrier particles have a larger particle size than the therapeutic agent to prevent the excipient from entering the airways. Segregation of the two particles will occur when turbulent airflow is created upon patient inhalation through the mouthpiece. This turbulence of inspiration will provide a certain amount of energy to overcome the interparticulate cohesive and particle surface adhesive forces for the micronized particles to become airborne. High concentrations of drug

particles in air are easily attained using dry powder generation, but stability of the output and the presence of agglomerated and charged particles are common problems. With very small particles, dispersion is difficult because of electrostatic, van der Waals, capillary, and mechanical forces that increase their energy of association.

An example of a dry powder inhaler aerosol generator suitable for use with the present invention is the Spinhaler powder inhaler available from Fisons Corp., Bedford, Massachusetts.

The FcRn molecule now is well characterized. As mentioned above, the FcRn has been isolated for several mammalian species, including humans. The FcRn occurs as a heterodimer involving an FcRn alpha chain (equivalently, FcRn heavy chain) and β_2 microglobulin. The sequence of the human FcRn, rat FcRn, and mouse FcRn alpha chains may be found in Story CM et al. (1994) *J Exp Med* 180:2377-81, which is incorporated herein by reference in its entirety. As will be recognized by those of ordinary skill in the art, FcRn can be isolated by cloning or by affinity purification using, for example, nonspecific antibodies, polyclonal antibodies, or monoclonal antibodies. Such isolated FcRn then can be used to identify and isolate FcRn binding partners, as described below.

The region of the Fc portion of IgG that binds to the FcRn has been described based upon X-ray crystallography (see, e.g., Burmeister WP et al (1994) *Nature* 372:379-83, and Martin WL et al. (2001) *Mol Cell* 7:867-77) which are incorporated by reference in their entirety). The major contact area of Fc with the FcRn is near the junction of the C_H2 and C_H3 domains. Potential IgG contacts are residues 248, 250-257, 272, 285, 288, 290-291, 307, 308-311 and 314 in C_H2 and 385-387, 428 and 433-436 in C_H3. These sites are distinct from those identified by subclass comparison or by site-directed mutagenesis as important for Fc binding to leukocyte FcγRI and FcγRII. Previous studies have implicated murine IgG residues 253, 272, 285, 310, 311, and 433-436 as potential contacts with FcRn. Shields RL et al. (2001) *J Biol Chem* 276:6591-6604. In the human IgG1, a previous study has implicated residues 253-256, 288, 307, 311, 312, 380, 382, and 433-436 as potential contacts with FcRn. Shields RL et al. (2001) *J Biol Chem* 276:6591-6604. The foregoing Fc - FcRn contacts are all within a single Ig heavy chain. It has been noted previously that two FcRn can bind a single Fc homodimer. The crystallographic data suggest that in such a complex, each FcRn molecule has major contacts with one polypeptide of the Fc homodimer. Martin WL et al. (1999) *Biochemistry* 39:9698-708.

Human FcRn binds to all subclasses of human IgG but not as well to most subclasses of mouse and rat IgG. West AP et al. (2000) *Biochemistry* 39:9698-9708; Ober RJ et al. (2001) *Int Immunol* 13:1551-59. Thus for a particular species there will be preferred species of IgG from which FcRn binding partners may be derived. The order of affinities of binding within each species is IgG1=IgG2>IgG3>IgG4 (human); IgG1>IgG2b>IgG2a>IgG3 (mouse); and IgG2a>IgG1>IgG2b=IgG2c (rat). Burmeister WP et al (1994) *Nature* 372:379-83. It is believed, therefore, that human IgG (and FcRn contact-containing fragments thereof) belonging to any subclass is useful as a human FcRn binding partner.

In an embodiment of the present invention, FcRn binding partners other than whole IgG may be used to transport therapeutics across the pulmonary epithelial barrier. In such an embodiment, it is preferred that an FcRn binding partner is chosen which binds the FcRn with higher affinity than whole IgG. Such an FcRn binding partner has utility in utilizing the FcRn to achieve active transport of a conjugated therapeutic across the epithelial barrier, and in reducing competition for the transport mechanism by endogenous IgG. The FcRn-binding activity of these higher affinity FcRn binding partners may be measured using standard assays known to those skilled in the art, including: (a) transport assays using polarized cells that naturally express the FcRn, or have been genetically engineered to express the FcRn or the alpha chain of the FcRn; (b) FcRn ligand:protein binding assays using soluble FcRn or fragments thereof, or immobilized FcRn; (c) binding assays utilizing polarized or non-polarized cells that naturally express the FcRn, or have been genetically engineered to express the FcRn or the alpha chain of the FcRn.

The FcRn binding partner may be produced by recombinant genetic engineering techniques. Within the scope of the invention are nucleotide sequences encoding human FcRn binding partners. The FcRn binding partners include whole IgG, the Fc fragment of IgG and other fragments of IgG that include the complete binding region for the FcRn. The major contact sites include amino acid residues 248, 250-257, 272, 285, 288, 290-291, 308-311 and 314 of the C_H2 domain and amino acid residues 385-387, 428 and 433-436 of the C_H3 domain. Therefore in a preferred embodiment of the present invention are nucleotide sequences encoding regions of the IgG Fc fragment spanning these amino acid residues.

The Fc region of IgG can be modified according to well recognized procedures such as site-directed mutagenesis and the like to yield modified IgG or modified Fc fragments or portions thereof that will be bound by the FcRn. Such modifications include modifications

remote from the FcRn contact sites as well as modifications within the contact sites that preserve or even enhance binding to the FcRn. For example, the following single amino acid residues in human IgG1 Fc (Fcγ1) can be substituted without significant loss of Fc binding affinity for FcRn: P238A, S239A, K246A, K248A, D249A, M252A, T256A, E258A, T260A, D265A, S267A, H268A, E269A, D270A, E272A, L274A, N276A, Y278A, D280A, V282A, E283A, H285A, N286A, T289A, K290A, R292A, E293A, E294A, Q295A, Y296F, N297A, S298A, Y300F, R301A, V303A, V305A, T307A, L309A, Q311A, D312A, N315A, K317A, E318A, K320A, K322A, S324A, K326A, A327Q, P329A, A330Q, P331A, E333A, K334A, T335A, S337A, K338A, K340A, Q342A, R344A, E345A, Q347A, R355A, E356A, M358A, T359A, K360A, K360A, N361A, Q362A, Y373A, S375A, D376A, A378Q, E380A, E382A, S383A, N384A, Q386A, E388A, N389A, N390A, Y391F, K392A, L398A, S400A, D401A, D413A, K414A, R416A, Q418A, Q419A, N421A, V422A, S424A, E430A, N434A, T437A, Q438A, K439A, S440A, S444A, and K447A, where for example P238A represents wildtype proline at position 238 substituted by alanine. Shields RL et al. (2001) *J Biol Chem* 276:6591-6604. Many but not all of the variants listed above are alanine variants, i.e., the wildtype residue is replaced by alanine. In addition to alanine, however, other amino acids may be substituted for the wildtype amino acids at the positions specified above. These mutations may be introduced singly into Fc, giving rise to more than one hundred FcRn binding partners structurally distinct from native human Fcγ1. Furthermore, combinations of two, three, or more of these individual mutations may be introduced together, giving rise to yet additional FcRn binding partners.

Certain of the above mutations may confer new functionality upon the FcRn binding partner. For example, a preferred embodiment incorporates N297A, removing a highly conserved N-glycosylation site. The effect of this mutation is to reduce immunogenicity, thereby enhancing circulating half-life of the FcRn binding partner, and to render the FcRn binding partner essentially incapable of binding to FcγRI, FcγRIIA, FcγRIIB, and FcγRIIIA, without compromise of its affinity for FcRn. Routledge EG et al. (1995) *Transplantation* 60:847-53; Friend PJ et al. (1999) *Transplantation* 68:1632-37;Shields RL et al. (2001) *J Biol Chem* 276:6591-6604. As a further example of new functionality arising from mutations above, affinity for FcRn may be increased beyond that of wildtype in some instances. This increased affinity may reflect an increased "on" rate, a decreased "off" rate, or both an increased "on" rate and a decreased "off" rate. Mutations believed may impart an increased

affinity for FcRn include in particular T256A, T307A, E380A, and N434A. Shields RL et al. (2001) *J Biol Chem* 276:6591-6604. Combination variants believed may impart an increased affinity for FcRn include in particular E380A/N434A, T307A/E380A/N434A, and K288A/N434A. Shields RL et al. (2001) *J Biol Chem* 276:6591-6604.

In addition to the FcRn binding partners disclosed above, in one embodiment, the FcRn binding partner is a polypeptide including the sequence: PKNSSMISNTP (SEQ ID NO:11), and optionally further including a sequence selected from the group consisting of HQSLGTQ (SEQ ID NO:12), HQNLSDGK (SEQ ID NO:13), HQNISDGK (SEQ ID NO:14), or VISSHLGQ (SEQ ID NO:15). U.S. Patent No. 5,739,277 issued to Presta et al. The sequence PKNSSMISNTP (SEQ ID NO:11) is to be compared with the sequence PKDTLMISRTP (SEQ ID NO:16) corresponding to amino acids 247-257 in the C_H2 domain of Fc (SEQ ID NO:2). The latter sequence encompasses nine amino acids previously noted to be believed to be major contact sites with FcRn.

It is not intended that the invention be limited by the selection of any particular FcRn binding partner. Thus, in addition to the FcRn binding partners just described, other binding partners can be identified and isolated. Antibodies or portions thereof specific for the FcRn and capable of being transported by FcRn once bound can be identified and isolated using well established techniques. Likewise, randomly generated molecularly diverse libraries can be screened and molecules that are bound and transported by FcRn can be isolated using conventional techniques. FcRn binding partners incorporating modifications to the polypeptide (i.e., polyamide) backbone, as distinguished from substitutions of the amino acid side chain groups, are also contemplated by the invention. For example, Bartlett et al. reported phosphonate-, phosphinate- and phosphinamide-containing pseudopeptide inhibitors of pepsin and penicillopepsin. Bartlett et al. (1990) *J Org Chem* 55:6268-74. See also U.S. Patent No. 5,563,121. Those inhibitors were pseudopeptides that included a phosphorus-containing bond in place of the scissile amide bond that would normally be cleaved by those enzymes.

In vitro screening methods for identifying and characterizing FcRn binding partners may be based on techniques familiar to those of skill in the art. These may include enzyme-linked immunosorbent assay (ELISA), where isolated FcRn is bound, directly or indirectly, to a substrate as a "capture antigen" and subsequently exposed to a sample containing a test FcRn binding partner; binding of the test FcRn binding partner to the immobilized FcRn is

then assayed directly or indirectly. In related methods, competitive ELISA or direct radioimmunoassay (RIA) may be used to determine affinity of an unlabeled test FcRn binding partner for FcRn relative to the affinity of a labeled standard FcRn binding partner for FcRn. These techniques are readily scalable and therefore suitable for large-scale and high throughput screening of candidate FcRn binding partners.

Additional in vitro screening methods useful for identifying and characterizing FcRn binding partners may be cell-based. These methods measure cell binding, cell uptake, or cell transcytosis of the test FcRn binding partner. Such methods may be facilitated by labeling the FcRn binding partner with, for example, an isotope (^{131}I, ^{35}S, ^{32}P, ^{13}C, etc.), a chromophore, a fluorophore, biotin, or an epitope recognized by an antibody (e.g., FLAG peptide). The cells used in these assays may express FcRn either naturally or as a result of introduction into the cells of an isolated nucleic acid molecule encoding FcRn, operatively linked to a suitable regulatory sequence. Typically the nucleic acid encoding FcRn, operatively linked to a suitable regulatory sequence, is a plasmid that is used to transform or transfect a host cell. Methods for transient and stable transformation and transfection are well known in the art, and they include physical, chemical, and viral techniques, for example calcium phosphate precipitation, electroporation, biolistic injection, and others.

Yet other in vitro methods suitable for identifying and characterizing FcRn binding partners may include flow cytometry (FACS), electromobility shift assay (EMSA), surface plasmon resonance (biomolecular interaction analysis; BIAcore), chip-based surface interaction analysis, and others.

If the FcRn binding partner is a peptide composed entirely of gene-encoded amino acids, or a portion of it is so composed, the peptide or the relevant portion may also be synthesized using conventional recombinant genetic engineering techniques. For recombinant production, a polynucleotide sequence encoding the FcRn binding partner is inserted into an appropriate expression vehicle, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence, or in the case of an RNA viral vector, the necessary elements for replication and translation. The expression vehicle is then transfected or otherwise introduced into a suitable target cell which will express the peptide. Depending on the expression system used, the expressed peptide is then isolated by procedures well-established in the art. Methods for recombinant protein and peptide production are well known in the art (see, e.g., Maniatis et al., 1989, Molecular

Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, New York).

To increase efficiency of production, the polynucleotide can be designed to encode multiple units of the FcRn binding partner separated by enzymatic cleavage sites. The resulting polypeptide can be cleaved (e.g., by treatment with the appropriate enzyme) in order to recover the peptide units. This can increase the yield of peptides driven by a single promoter. When used in appropriate viral expression systems, the translation of each peptide encoded by the mRNA is directed internally in the transcript, e.g., by an internal ribosome entry site, IRES. Thus, the polycistronic construct directs the transcription of a single, large polycistronic mRNA which, in turn, directs the translation of multiple, individual peptides. This approach eliminates the production and enzymatic processing of polyproteins and may significantly increase yield of peptide driven by a single promoter.

A variety of host-expression vector systems may be utilized to express the FcRn binding partners described herein. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage DNA or plasmid DNA expression vectors containing an appropriate coding sequence; yeast or filamentous fungi transformed with recombinant yeast or fungi expression vectors containing an appropriate coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing an appropriate coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus (CaMV) or tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing an appropriate coding sequence; or animal cell systems. Various host-expression systems are well known by those of skill in the art, and the host cell and expression vector elements are available from commercial sources.

The expression elements of the expression systems vary in their strength and specificities. Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used in the expression vector. For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage λ, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used; when cloning in insect cell systems, promoters such as the baculovirus polyhedron promoter may be used; when cloning in plant cell systems, promoters derived

from the genome of plant cells (e.g., heat shock promoters; the promoter for the small subunit of RUBISCO; the promoter for the chlorophyll a/b binding protein) or from plant viruses (e.g., the 35S RNA promoter of CaMV; the coat protein promoter of TMV) may be used; when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5 K promoter; the cytomegalovirus (CMV) promoter) may be used; when generating cell lines that contain multiple copies of expression product, SV40-, BPV- and EBV-based vectors may be used with an appropriate selectable marker.

In cases where plant expression vectors are used, the expression of sequences encoding the polypeptides of the invention may be driven by any of a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV (Koziel MG et al. (1984) *J Mol Appl Genet* 2:549-62), or the coat protein promoter of TMV may be used; alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi G et al. (1984) *EMBO J* 3:1671-79; Broglie R et al. (1984) *Science* 224:838-43) or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley WB et al. (1986) *Mol Cell Biol* 6:559-65) may be used. These constructs can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, microinjection, electroporation, etc. For reviews of such techniques see, e.g., Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp. 421-463; and Grierson & Corey, 1988, Plant Molecular Biology, 2d Ed., Blackie, London, Ch. 7-9.

In one insect expression system that may be used to express the FcRn binding partners, *Autographa californica* nuclear polyhidrosis virus (AcNPV) is used as a vector to express the foreign genes. The virus grows in *Spodoptera frugiperda* cells. A coding sequence may be cloned into non-essential regions (for example the polyhedron gene) of the virus and placed under control of an AcNPV promoter (for example, the polyhedron promoter). Successful insertion of a coding sequence will result in inactivation of the polyhedron gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedron gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed (e.g., see U.S. Patent No. 4,745,051). Further examples of this expression system may be found in Current Protocols in Molecular Biology, Vol. 2, Ausubel et al., eds., Greene Publishing Associates and Wiley Interscience, N.Y.

In mammalian host cells, a number of viral based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a coding sequence may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing peptide in infected hosts (see, e.g., Logan J et al. (1984) *Proc Natl Acad Sci USA* 81:3655-59). Alternatively, the vaccinia 7.5 K promoter may be used, (see, e.g., Mackett M et al. (1982) *Proc Natl Acad Sci USA* 79:7415-19; Mackett M et al. (1984) *J Virol* 49:857-64; Panicali S et al. (1982) *Proc Natl Acad Sci USA* 79:4927-31).

Also for use in mammalian host cells are a number of eukaryotic expression plasmids. These plasmids typically include a promoter or promoter/enhancer element operably linked to the inserted gene or nucleic acid of interest, a polyadenylation signal positioned downstream of the inserted gene, a selection marker, and an origin of replication. Some of these plasmids are designed to accept nucleic acid inserts at specified positions, either as PCR products or as restriction enzyme digest products. Examples of eukaryotic expression plasmids include pRc/CMV, pcDNA3.1, pcDNA4, pcDNA6, pGene/V5 (Invitrogen), and pED.dC (Genetics Institute).

The FcRn binding partner is in some embodiments conjugated with an antigen. An antigen as used herein falls into four classes: (1) antigens that are characteristic of a pathogen; (2) antigens that are characteristic of an autoimmune disease; (3) antigens that are characteristic of an allergen; and (4) antigens that are characteristic of a cancer or tumor. Antigens in general include polysaccharides, glycolipids, glycoproteins, peptides, proteins, carbohydrates and lipids from cell surfaces, cytoplasm, nuclei, mitochondria and the like.

Antigens that are characteristic of pathogens include antigens derived from viruses, bacteria, parasites or fungi. Examples of important pathogens include *Vibrio cholerae*, enterotoxigenic *Escherichia coli*, rotavirus, *Clostridium difficile*, *Shigella* species, *Salmonella typhi*, parainfluenza virus, influenza virus, *Streptococcus pneumoniae*, *Borrelia burgdorferi*, HIV, *Streptococcus mutans*, *Plasmodium falciparum*, *Staphylococcus aureus*, rabies virus and Epstein-Barr virus.

Viruses in general include but are not limited to those in the following families: picornaviridae; caliciviridae; togaviridae; flaviviridae; coronaviridae; rhabdoviridae;

filoviridae; paramyxoviridae; orthomyxoviridae; bunyaviridae; arenaviridae; reoviridae; retroviridae; hepadnaviridae; parvoviridae; papovaviridae; adenoviridae; herpesviridae; and poxviridae.

Bacteria in general include but are not limited to: *Pseudomonas* spp., including *P. aeruginosa and P. cepacia*; *Escherichia* spp., including *E. coli*, *E. faecalis*; *Klebsiella* spp.; *Serratia* spp.; *Acinetobacter* spp.; *Streptococcus* spp., including *S. pneumoniae*, *S. pyogenes*, *S. bovis*, *S. agalactiae*; *Staphylococcus* spp., including *S. aureus*, *S. epidermidis*; *Haemophilus* spp.; *Neisseria* spp., including *N. meningitidis*; *Bacteroides* spp.; *Citrobacter* spp.; *Branhamella* spp.; *Salmonella* spp.; *Shigella* spp.; *Proteus* spp., including *P. mirabilis*; *Clostridium* spp.; *Erysipelothrix* spp.; *Listeria* spp.; *Pasteurella multocida*; *Streptobacillus spp.*; *Spirillum* spp.; *Fusospirocheta* spp.; *Treponema pallidum*; *Borrelia* spp.; *Actinomycetes*; *Mycoplasma* spp.; *Chlamydia* spp.; *Rickettsia* spp.; *Spirochaeta*; *Legionella* spp.; *Mycobacteria* spp., including *M. tuberculosis*, *M. kansasii*, *M. intracellulare*, *M. marinum*; *Ureaplasma* spp.; *Streptomyces* spp.; and *Trichomonas* spp.

Parasites include but are not limited to: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malaria*; *Toxoplasma gondii*; *Leishmania mexicana*, *L. tropica*, *L. major*, *L. aethiopica*, *L. donovani*, *Trypanosoma cruzi*, *T. brucei*, *Schistosoma mansoni*, *S. haematobium*, *S. japonium*; *Trichinella spiralis*; *Wuchereria bancrofti*; *Brugia malayi*; *Entamoeba histolytica*; *Enterobius vermicularis*; *Taenia solium*, *T. saginata*, *Trichomonas vaginalis*, *T. hominis*, *T. tenax*; *Giardia lamblia*; *Cryptosporidium parvum*; *Pneumocystis carinii*, *Babesia bovis*, *B. divergens*, *B. microti*, *Isospora belli*, *L. hominis*; *Dientamoeba fragilis*; *Onchocerca volvulus*; *Ascaris lumbricoides*; *Necator americanis*; *Ancylostoma duodenale*; *Strongyloides stercoralis*; *Capillaria philippinensis*; *Angiostrongylus cantonensis*; *Hymenolepis nana*; *Diphyllobothrium latum*; *Echinococcus granulosus*, *E. multilocularis*; *Paragonimus westermani*, *P. caliensis*; *Chlonorchis sinensis*; *Opisthorchis felineas*, *G. viverini*, *Fasciola hepatica*, *Sarcoptes scabiei*, *Pediculus humanus*; *Phthirlus pubis*; and *Dermatobia hominis*.

Fungi in general include but are not limited to: *Cryptococcus neoformans*; *Blastomyces dermatitidis*; *Aiellomyces dermatitidis*; *Histoplasma capsulatum*; *Coccidioides immitis*; *Candida* species, including *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii* and *C. krusei*; *Aspergillus* species, including *A. fumigatus*, *A. flavus* and *A. niger*; *Rhizopus* species; *Rhizomucor* species; *Cunninghammella* species; *Apophysomyces* species, including *A. saksenaea*, *A. mucor* and *A. absidia*; *Sporothrix schenckii*;

Paracoccidioides brasiliensis; *Pseudallescheria boydii*; *Torulopsis glabrata*; and *Dermatophytes* species.

Antigens that are characteristic of autoimmune disease typically will be derived from the cell surface, cytoplasm, nucleus, mitochondria and the like of mammalian tissues. Examples include antigens characteristic of uveitis (e.g., S antigen), diabetes mellitus, multiple sclerosis, systemic lupus erythematosus, Hashimoto's thyroiditis, myasthenia gravis, primary myxoedema, thyrotoxicosis, rheumatoid arthritis, pernicious anemia, Addison's disease, scleroderma, autoimmune atrophic gastritis, premature menopause (few cases), male infertility (few cases), juvenile diabetes, Goodpasture's syndrome, pemphigus vulgaris, pemphigoid, sympathetic ophthalmia, phacogenic uveitis, autoimmune haemolytic anemia, idiopathic thrombocytopenic purpura, idiopathic leukopenia, primary biliary cirrhosis (few cases), ulcerative colitis, Sjögren's syndrome, Wegener's granulomatosis, poly/dermatomyositis, and discoid lupus erythematosus. It is to be understood that an antigen characteristic of autoimmune disease refers to an antigen against which a subject's own immune system makes antibodies or specific T cells, and those antibodies or T cells are characteristic of an autoimmune disease. The specific identity of an antigen characteristic of an autoimmune disease in many cases is not, and indeed for the purposes of the invention need not, be known.

Antigens that are allergens are generally proteins or glycoproteins, although allergens may also be low molecular weight allergenic haptens that induce allergy after covalently combining with a protein carrier (Remington's Pharmaceutical Sciences). Allergens include antigens derived from pollens, dust, molds, spores, dander, insects and foods. Specific examples include the urushiols (pentadecylcatechol or heptadecyicatechol) of Toxicodendron species such as poison ivy, poison oak and poison sumac, and the sesquiterpenoid lactones of ragweed and related plants.

Antigens that are characteristic of tumor antigens typically will be derived from the cell surface, cytoplasm, nucleus, organelles and the like of cells of tumor tissue. Examples include antigens characteristic of tumor proteins, including proteins encoded by mutated oncogenes; viral proteins associated with tumors; and tumc_ mucins and glycolipids. Tumors include, but are not limited to, those from the following sites of cancer and types of cancer: lip, nasopharynx, pharynx and oral cavity, esophagus, stomach, small intestine, colon, rectum, liver, gall bladder, biliary tree, pancreas, larynx, lung and bronchus, melanoma, breast, cervix,

uterus, ovary, bladder, kidney, brain and other parts of the nervous system, thyroid, prostate, testes, bone, muscle, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma and leukemia. Viral proteins associated with tumors would be those from the classes of viruses noted above. An antigen characteristic of a tumor may be a protein not usually expressed by a tumor precursor cell, or may be a protein which is normally expressed in a tumor precursor cell, but having a mutation characteristic of a tumor. An antigen characteristic of a tumor may be a mutant variant of the normal protein having an altered activity or subcellular distribution. Mutations of genes giving rise to tumor antigens, in addition to those specified above, may be in the coding region, 5' or 3' noncoding regions, or introns of a gene, and may be the result of point mutations, frameshifts, inversions, deletions, additions, duplications, chromosomal rearrangements and the like. One of ordinary skill in the art is familiar with the broad variety of alterations to normal gene structure and expression which gives rise to tumor antigens.

Specific examples of tumor antigens include: proteins such as Ig-idiotype of B cell lymphoma; mutant cyclin-dependent kinase 4 of melanoma; Pmel-17 (gp 100) of melanoma; MART-1 (Melan-A) of melanoma (PCT publication WO94/21126); p15 protein of melanoma; tyrosinase of melanoma (PCT publication WO94/14459); MAGE 1, 2 and 3 of melanoma, thyroid medullary, small cell lung cancer, colon and/or bronchial squamous cell cancer (PCT/US92/04354); MAGE-Xp (U.S. Patent No. 5,587,289); BAGE of bladder, melanoma, breast, and squamous-cell carcinoma (U.S. Patent No. 5,571,711 and PCT publication WO95/00159); GAGE (U.S. Patent No. 5,610,013 and PCT publication WO95/03422); RAGE family (U.S. Patent No. 5,939,526); PRAME (formerly DAGE; PCT publication WO96/10577); MUM-1/LB-33B (U.S. Patent No. 5,589,334); NAG (U.S. Patent No. 5,821,122); FB5 (endosialin) (U.S. Patent No. 6,217,868); PSMA (prostate-specific membrane antigen;U.S. Patent No. 5,935,818); gp75 of melanoma; oncofetal antigen of melanoma; carbohydrate/lipids such as mucin of breast, pancreas, and ovarian cancer; GM2 and GD2 gangliosides of melanoma; oncogenes such as mutant p53 of carcinoma; mutant ras of colon cancer; HER2/neu proto-oncogene of breast carcinoma; and viral products such as human papilloma virus proteins of squamous cell cancers of cervix and esophagus. The foregoing list is only intended to be representative and is not to be understood to be limiting. It is also contemplated that proteinaceous tumor antigens may be presented by HLA molecules as specific peptides derived from the whole protein. Metabolic processing of

proteins to yield antigenic peptides is well known in the art (see, e.g., U.S. Patent No. 5,342,774, issue to Boon et al., which is incorporated herein by reference in its entirety). The present method thus encompasses delivery of antigenic peptides and such peptides in a larger polypeptide or whole protein which give rise to antigenic peptides. Delivery of antigenic peptides or proteins may give rise to humoral or cellular immunity.

Generally, subjects can receive an effective amount of an antigen, including a tumor antigen, and/or a peptide derived therefrom, by one or more of the methods detailed below. Initial doses can be followed by booster doses, following immunization protocols standard in the art. Delivery of antigens, including tumor antigens, thus may stimulate proliferation of cytolytic T lymphocytes.

In the cases of protein and peptide therapeutic agents, covalent linking to an FcRn binding partner is intended to include linkage by peptide bonds in a single polypeptide chain. Established methods (Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY 1989, which is incorporated herein by reference in its entirety) would be used to engineer DNA encoding a fusion protein comprised of the protein or peptide therapeutic agent and an FcRn binding partner. This DNA would be placed in an expression vector and introduced into bacterial, eukaryotic, or other suitable host cells by established methods. The fusion protein would be purified from the cells or from the culture medium by established methods. The purification scheme may conveniently use isolated or recombinant protein A or protein G to purify FcRn binding partner-containing fusion proteins from host cell products. Such resulting conjugates include fusions of the FcRn binding partner to a protein, peptide or protein derivative such as those listed herein including, but not limited to, antigens, allergens, pathogens or to other proteins or protein derivatives of potential therapeutic interest such as growth factors, colony stimulating factors, growth inhibitory factors, signaling molecules, hormones, steroids, neurotransmitters, or morphogens that would be of use when delivered across an epithelial barrier.

By way of example, but not limitation, proteins used in fusion proteins to synthesize conjugates may include EPO (U.S. Patent Nos. 4,703,008; 5,457,089; 5,614,184; 5,688,679; 5,773,569; 5,856,298; 5,888,774; 5,986,047; 6,048,971; 6,153,407), IFN-α (U.S. Patent Nos. 4,678,751; 4,801,685; 4,820,638; 4,921,699; 4,973,479; 4,975,276; 5,098,703; 5,310,729; 5,869,293; 6,300,474), IFN-β (U.S. Patent Nos. 4,820,638; 5,460,811), FSH (U.S. Patent Nos. 4,923,805; 5,338,835; 5,639,639; 5,639,640; 5,767,251; 5,856,137), platelet-derived

growth factor (PDGF; U.S. Patent No. 4,766,073), platelet-derived endothelial cell growth factor (PD-ECGF; U.S. Patent No. 5,227,302), human pituitary growth hormone (hGH; U.S. Patent No. 3,853,833), TGF-β (U.S. Patent No. 5,168,051), TGF-α (U.S. Patent No. 5,633,147), keratinocyte growth factor (KGF; U.S. Patent No. 5,731,170), insulin-like growth factor I (IGF-I; U.S. Patent No. 4,963,665), epidermal growth factor (EGF; U.S. Patent No. 5,096,825), granulocyte-macrophage colony-stimulating factor (GM-CSF; U.S. Patent No. 5,200,327), macrophage colony-stimulating factor (M-CSF; U.S. Patent No. 5,171,675), colony stimulating factor-1 (CSF-1; U.S. Patent No. 4,847,201), Steel factor, Calcitonin, AP-1 proteins (U.S. Patent No. 5,238,839), Factor VIIa, Factor VIII, Factor IX, TNF-α, TNF-α receptor, LFA-3, CNTF, CTLA-4, leptin (PCT/US95/10479, WO 96/05309), and brain-derived neurotrophic factor (BDNF; U.S. Patent No. 5,229,500). All of the references cited above are incorporated herein by reference in their entirety.

By way of example, but not limitation, peptides used in fusion proteins to synthesize conjugates may include erythropoietin mimetic peptides (EPO receptor agonist peptides; PCT/US01/14310; WO 01/83525; Wrighton NC et al. (1996) *Science* 273:458-64; PCT/US99/05842, WO 99/47151), EPO receptor antagonist peptides (PCT/US99/05842, WO 99/47151; McConnell SJ et al. (1998) *Biol Chem* 379:1279-86), and T20 (PCT/US00/35724; WO 01/37896).

In a preferred embodiment, the fusion proteins of the invention are constructed and arranged so that the FcRn binding partner portion of the conjugate occurs downstream of the therapeutic agent portion, i.e., the FcRn binding partner portion is C-terminal with respect to the therapeutic agent portion. This arrangement is expressed in a short-hand manner as X-Fc, where "X" represents the therapeutic agent portion and Fc represents the FcRn binding partner portion. In this short-hand notation, "Fc" may be, but is not limited to, Fc fragment of IgG. The notation "X-Fc" is to be understood to encompass fusion proteins in which is present a linker joining the X and FcRn binding partner components.

In one embodiment, fusion proteins of the present invention are constructed in which the conjugate consists of an Fc fragment of human IgG1 (starting with the amino acids D-K-T-H at the N-terminus of the hinge (see SEQ ID NO:2, **Figure 1**), including the hinge and C_H2 domain, and continuing through the S-P-G-K sequence in the C_H3 domain) fused to one of the polypeptide therapeutic agents listed herein. In one preferred embodiment, a nucleotide sequence encoding functional EPO is fused in proper translational reading frame 5' to a

nucleotide sequence encoding the hinge, C_H2 domain, and C_H3 domain of the constant heavy (C_H) chain of human IgG1. This preferred embodiment is described in more detail in Example 3.

Published European patent application EP 0 464 533 A discloses an EPO-Fc fusion protein.

Published PCT application PCT/US00/19336 (WO 01/03737) discloses a human EPO-Fc fusion protein.

Published PCT application PCT/US98/13930 (WO 99/02709) discloses EPO-Fc and Fc-EPO fusion proteins.

Published PCT application PCT/EP00/10843 (WO 01/36489) discloses a number of Fc-EPO fusion proteins.

Published PCT application PCT/US00/19336 (WO 01/03737) discloses a human IFN-α-Fc fusion protein.

U.S. Patent No. 5,723,125 issued to Chang et al. discloses a human IFN-α-Fc fusion protein wherein the IFN-α and Fc domains are connected through a particular Gly-Ser linker (Gly-Gly-Ser-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser; SEQ ID NO:17).

Published PCT application PCT/US00/13827 (WO 00/69913) discloses an Fc-IFN-α fusion protein.

Published PCT application PCT/US00/19336 (WO 01/03737) discloses a human IFN-β-Fc fusion protein.

Published PCT application PCT/US99/24200 (WO 00/23472) discloses a human IFN-β-Fc fusion protein.

U.S. Patent No. 5,908,626 issued to Chang et al. discloses a human IFN-β-Fc fusion protein wherein the IFN-β and Fc domains are connected through a particular Gly-Ser linker (Gly-Gly-Ser-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser; SEQ ID NO:17).

U.S. Patent No. 5,726,044 issued to Lo et al., and published PCT application PCT/US00/19816 (WO 01/07081), discloses an Fc-PSMA fusion construct.

The FcRn binding partners may be conjugated to a variety of therapeutic agents for targeted systemic delivery. The present invention encompasses the targeted systemic delivery of biologically active substances.

As used herein, the term "biologically active substance" refers to eukaryotic and prokaryotic cells, viruses, vectors, proteins, peptides, nucleic acids, polysaccharides and carbohydrates, lipids, glycoproteins, and combinations thereof, and naturally-occurring, synthetic, and semi-synthetic organic and inorganic drugs exerting a biological effect when administered to an animal. For ease of reference, the term is also used to include detectable compounds such as radio-opaque compounds including barium, as well as magnetic compounds. The biologically active substance can be soluble or insoluble in water. Examples of biologically active substances include anti-angiogenesis factors, antibodies, growth factors, hormones, enzymes, and drugs such as steroids, anti-cancer drugs and antibiotics.

In diagnostic embodiments, the FcRn binding partners may also be conjugated to a pharmaceutically acceptable gamma-emitting moiety, including but not limited to, indium and technetium, magnetic particles, radio-opaque materials such as barium, and fluorescent compounds.

By way of example, and without limitation, the following classes of drugs may be conjugated to FcRn binding partners for the purposes of systemic delivery across pulmonary epithelial barrier:

Antineoplastic Compounds. Nitrosoureas, e.g., carmustine, lomustine, semustine, strepzotocin; Methylhydrazines, e.g., procarbazine, dacarbazine; steroid hormones, e.g., glucocorticoids, estrogens, progestins, androgens, tetrahydrodesoxycaricosterone, cytokines and growth factors; Asparaginase.

Immunoactive Compounds. Immunosuppressives, e.g., pyrimethamine, trimethopterin, penicillamine, cyclosporine, azathioprine; immunostimulants, e.g., levamisole, diethyl dithiocarbamate, enkephalins, endorphins.

Antimicrobial Compounds. Antibiotics, e.g., penicillins, cephalosporins, carbapenims and monobactams, β-lactamase inhibitors, aminoglycosides, macrolides, tetracyclins, spectinomycin; Antimalarials; Amebicides; Antiprotazoal agents; Antifungal agents, e.g., amphotericin B; Antiviral agents, e.g., acyclovir, idoxuridine, ribavirin, trifluridine, vidarabine, gancyclovir.

Gastrointestinal Drugs. Histamine H_2 receptor antagonists, proton pump inhibitors, promotility agents.

Hematologic Compounds. Immunoglobulins; blood clotting proteins; e.g., antihemophiliac factor, factor IX complex; anticoagulants, e.g., dicumarol, heparin Na; fibrolysin inhibitors, tranexamic acid.

Cardiovascular Drugs. Peripheral antiadrenergic drugs, centrally acting antihypertensive drugs, e.g., methyldopa, methyldopa HCl; antihypertensive direct vasodilators, e.g., diazoxide, hydralazine HCl; drugs affecting renin-angiotensin system; peripheral vasodilators, phentolamine; antianginal drugs; cardiac glycosides; inodilators; e.g., amrinone, milrinone, enoximone, fenoximone, imazodan, sulmazole; antidysrhythmic; calcium entry blockers; drugs affecting blood lipids.

Neuromuscular Blocking Drugs. Depolarizing, e.g., atracurium besylate, hexafluorenium Br, metocurine iodide, succinylcholine Cl, tubocurarine Cl, vecuronium Br; centrally acting muscle relaxants, e.g., baclofen.

Neurotransmitters and Neurotransmitter Agents. Acetylcholine, adenosine, adenosine triphosphate, amino acid neurotransmitters, e.g., excitatory amino acids, GABA, glycine; biogenic amine neurotransmitters, e.g., dopamine, epinephrine, histamine, norepinephrine, octopamine, serotonin, tyramine; neuropeptides, nitric oxide, K+ channel toxins.

Antiparkinson Drugs. Amantidine HCl, benztropine mesylate, e.g., carbidopa.

Diuretic Drugs. Dichlorphenamide, methazolamide, bendroflumethiazide, polythiazide.

Antimigraine Drugs. Sumatriptan.

Hormones. Pituitary hormones, e.g., chorionic gonadotropin, cosyntropin, menotropins, somatotropin, iorticotropin, protirelin, thyrotropin, vasopressin, lypressin; adrenal hormones, e.g., beclomethasone dipropionate, betamethasone, dexamethasone, triamcinolone; pancreatic hormones, e.g., glucagon, insulin; parathyroid hormone, e.g., dihydrochysterol; thyroid hormones, e.g., calcitonin etidronate disodium, levothyroxine Na, liothyronine Na, liotrix, thyroglobulin, teriparatide acetate; antithyroid drugs; estrogenic hormones; progestins and antagonists, hormonal contraceptives, testicular hormones; gastrointestinal hormones: cholecystokinin, enteroglycan, galanin, gastric inhibitory polypeptide, epidermal growth factor-urogastrone, gastric inhibitory polypeptide, gastrin-releasing peptide, gastrins, pentagastrin, tetragastrin, motilin, peptide YY, secretin, vasoactive intestinal peptide, sincalide; leptin.

Enzymes. Hyaluronidase, streptokinase, tissue plasminogen activator, urokinase, PGE-adenosine deaminase.

Intravenous Anesthetics. Droperidol, etomidate, fentanyl citrate/droperidol, hexobarbital, ketamine HCl, methohexital Na, thiamylal Na, thiopental Na.

Antiepileptics. Carbamazepine, clonazepam, divalproex Na, ethosuximide, mephenytoin, paramethadione, phenytoin, primidone.

Peptides and Proteins. The FcRn binding partners may be conjugated to peptides or polypeptides, e.g., ankyrins, arrestins, bacterial membrane proteins, clathrin, connexins, dystrophin, endothelin receptor, spectrin, selectin, cytokines, chemokines, growth factors, insulin, erythropoietin (EPO), tumor necrosis factor (TNF), CNTF, neuropeptides, neuropeptide Y, neurotensin, TGF-α, TGF-β, interferon (IFN), and hormones, growth inhibitors, e.g., genistein, steroids etc; glycoproteins, e.g., ABC transporters, platelet glycoproteins, GPIb-IX complex, GPIIb-IIIa complex, Factor VIIa, Factor VIII, Factor IX, vitronectin, thrombomodulin, CD4, CD55, CD58, CD59, CD44, CD 152 (CTLA-4), lymphocye function-associated antigens (LFAs), intercellular adhesion molecules (ICAMs), vascular cell adhesion molecules (VCAMs), Thy-1, antiporters, CA-15-3 antigen, fibronectins, laminin, myelin-associated glycoprotein, GAP, GAP-43, and binding portions of receptors and counter-receptors for the above. In this embodiment of the present invention, the polypeptide therapeutics may be covalently conjugated to the FcRn binding partner, or the FcRn binding partner and therapeutic may be expressed as a fusion protein using standard recombinant genetic techniques.

Cytokines and Cytokine Receptors. Examples of cytokines and receptors thereof which may be delivered via an FcRn binding partner or conjugated to an FcRn binding partner in accordance with the present invention, include, but are not limited to: Interleukin-1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-1 receptor, IL-2 receptor, IL-3 receptor, IL-4 receptor, IL-5 receptor, IL-6 receptor, IL-7 receptor, IL-8 receptor, IL-9 receptor, IL-10 receptor, IL-11 receptor, IL-12 receptor, IL-13 receptor, IL-14 receptor, IL-15 receptor, IL-16 receptor, IL-17 receptor, IL-18 receptor, lymphokine inhibitory factor (LIF), M-CSF, PDGF, stem cell factor, transforming growth factor beta (TGF-β), TNF, TNFR, lymphotoxin, Fas, granulocyte colony-stimulating factor (G-CSF), GM-CSF, IFN-α, IFN-β, IFN-γ.

Growth Factors and Protein Hormones. Examples of growth factors and receptors thereof and protein hormones and receptors thereof which may be delivered via an FcRn binding partner or conjugated to an FcRn binding partner in accordance with the present invention, include, but are not limited to: EPO, angiogenin, hepatocyte growth factor, fibroblast growth factor, keratinocyte growth factor, nerve growth factor, tumor growth factor α, thrombopoietin (TPO), thyroid stimulating factor, thyroid releasing hormone, neurotrophin, epidermal growth factor, VEGF, ciliary neurotrophic factor, LDL, somatomedin, insulin growth factor, insulin-like growth factor I and II.

Chemokines. Examples of chemokines and receptors thereof which may be delivered via an FcRn binding partner or conjugated to an FcRn binding partner in accordance with the present invention, include, but are not limited to: ENA-78, ELC, GRO-α, GRO-β, GRO-γ, HRG, LIF, IP-10, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1α, MIP-1β, MIG, MDC, NT-3, NT-4, SCF, LIF, leptin, RANTES, lymphotactin, eotaxin-1, eotaxin-2, TARC, TECK, WAP-1, WAP-2, GCP-1, GCP-2, α-chemokine receptors: CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CXCR7, β-chemokine receptors: CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7.

Chemotherapeutics. The FcRn binding partners may be conjugated to chemotherapy or anti-tumor agents which are effective against various types of human and other cancers, including leukemia, lymphomas, carcinomas, sarcomas, myelomas etc., such as, doxorubicin, mitomycin, cisplatin, daunorubicin, bleomycin, actinomycin D, neocarzinostatin, vinblastine, vincristine, taxol.

Antiviral Agents. The FcRn binding partners may be conjugated to antiviral agents such as reverse transcriptase inhibitors and nucleoside analogs, e.g., ddI, ddC, 3TC, ddA, AZT; protease inhibitors, e.g., Invirase, ABT-538; inhibitors of in RNA processing, e.g., ribavirin; and inhibitors of cell fusion, e.g., T-20 (Kilby JM et al. (1998) *Nat Med.* 4:1302-7).

Nucleic Acids. The FcRn binding partners may be conjugated to nucleic acid molecules such as antisense oligonucleotides and gene replacement nucleic acids. In embodiments involving conjugates with nucleic acids, it is believed that it is preferable to include a cleavable linker between the nucleic acid and the FcRn binding partner so that the nucleic acid can be available intracellularly. Antisense oligonucleotides include, for example and without limitation, anti-PKC-α, anti-ICAM-1, anti-H-ras, anti-Raf, anti-TNF-α, anti-

VLA-4, anti-clusterin (all from Isis Pharmaceuticals, Inc.) and anti-Bcl-2 (GENASENSE™; Genta, Inc.).

Specific examples of known therapeutics which may be delivered via an FcRn binding partner include, but are not limited to:

(a) Capoten, Monopril, Pravachol, Avapro, Plavix, Cefzil, Duricef/Ultracef, Azactam, Videx, Zerit, Maxipime, VePesid, Paraplatin, Platinol, Taxol, UFT, Buspar, Serzone, Stadol NS, Estrace, Glucophage (Bristol-Myers Squibb);

(b) Ceclor, Lorabid, Dynabac, Prozac, Darvon, Permax, Zyprexa, Humalog, Axid, Gemzar, Evista (Eli Lilly);

(c) Vasotec/Vaseretic, Mevacor, Zocor, Prinivil/Prinizide, Plendil, Cozaar/Hyzaar, Pepcid, Prilosec, Primaxin, Noroxin, Recombivax HB, Varivax, Timoptic/XE, Trusopt, Proscar, Fosamax, Sinemet, Crixivan, Propecia, Vioxx, Singulair, Maxalt, Ivermectin (Merck & Co.);

(d) Diflucan, Unasyn, Sulperazon, Zithromax, Trovan, Procardia XL, Cardura, Norvasc, Dofetilide, Feldene, Zoloft, Zeldox, Glucotrol XL, Zyrtec, Eletriptan, Viagra, Droloxifene, Aricept, Lipitor (Pfizer);

(e) Vantin, Rescriptor, Vistide, Genotropin, Micronase/Glyn./Glyb., Fragmin, Total Medrol, Xanax/alprazolam, Sermion, Halcion/triazolam, Freedox, Dostinex, Edronax, Mirapex, Pharmorubicin, Adriamycin, Camptosar, Remisar, Depo-Provera, Caverject, Detrusitol, Estring, Healon, Xalatan, Rogaine (Pharmacia & Upjohn);

(f) Lopid, Accrupil, Dilantin, Cognex, Neurontin, Loestrin, Dilzem, Fempatch, Estrostep, Rezulin, Lipitor, Omnicef, FemHRT, Suramin, Clinafloxacin (Warner Lambert).

Further examples of therapeutic agents which may be delivered by the FcRn binding partners of the present invention may be found in Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th ed., McGraw-Hill 1996, incorporated herein by reference in its entirety.

When administered, the conjugates of the present invention are administered in pharmaceutically acceptable preparations. Such preparations may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines, and optionally other therapeutic agents. Thus, "cocktails" including the conjugates and the agents are contemplated. The therapeutic agents themselves are conjugated to FcRn

binding partners to enhance delivery of the therapeutic agents across the pulmonary epithelial barrier.

The conjugates of the invention may be administered in a purified form or in the form of a pharmaceutically acceptable salt. When used in medicine the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof and are not excluded from the scope of the invention. Such pharmaceutically acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulfonic, tartaric, citric, methane sulfonic, formic, malonic, succinic, naphthalene-2-sulfonic, and benzene sulfonic. Also, pharmaceutically acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

Suitable buffering agents include: acetic acid and salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2:5% w/v); sodium bicarbonate (0.5-1.0% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives include benzalkonium chloride (0.003-0.03% w/v); chlorbutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

The term "carrier" as used herein, and described more fully below, means one or more solid or liquid filler, dilutant or encapsulating substances which are suitable for administration to a human or other mammal. The "carrier" may be an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate administration.

The components of the pharmaceutical compositions are capable of being commingled with the conjugates of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy. In certain embodiments the components of aerosol formulations include solubilized active ingredients, and optionally antioxidants, solvent blends and propellants for solution formulations; micronized and suspended active ingredients, and optionally dispersing agents and propellants for suspension formulations.

The term "adjuvant" is intended to include any substance which is incorporated into or administered simultaneously with the conjugates of the invention and which nonspecifically potentiates the immune response in the subject. Adjuvants include aluminum compounds,

e.g., gels, aluminum hydroxide and aluminum phosphate, and Freund's complete or incomplete adjuvant (in which the conjugate is incorporated in the aqueous phase of a stabilized water in paraffin oil emulsion). The paraffin oil may be replaced with different types of oils, e.g., squalene or peanut oil. Other materials with adjuvant properties include BCG (attenuated *Mycobacterium bovis*), calcium phosphate, levamisole, isoprinosine, polyanions (e.g., poly A:U), leutinan, pertussis toxin, cholera toxin, lipid A, saponins and peptides, e.g., muramyl dipeptide. Rare earth salts, e.g., lanthanum and cerium, may also be used as adjuvants. The amount of adjuvants depends on the subject and the particular conjugate used and can be readily determined by one skilled in the art without undue experimentation.

Other supplementary immune potentiating agents, such as cytokines, may be delivered in conjunction with the conjugates of the invention. In one embodiment, cytokines are administered separately from conjugates of the invention in order to supplement treatment. In another embodiment, cytokines are administered conjugated to an FcRn binding partner. The cytokines contemplated are those that will enhance the beneficial effects that result from administering the FcRn binding partner conjugates according to the invention. Particularly preferred cytokines are IFN-α, IFN-β, IFN-γ, IL-1, IL-2, and TNF-α. Other useful cytokines and related molecules are believed to be IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-18, leukemia inhibitory factor, oncostatin-M, ciliary neurotrophic factor, growth hormone, prolactin, CD40 ligand, CD27 ligand, CD30 ligand, and TNF-β. Other cytokines known to modulate T-cell activity in a manner likely to be useful according to the invention are colony-stimulating factors and growth factors including granulocyte and/or granulocyte-macrophage colony-stimulating factors (CSF-1, G-CSF, and GM-CSF) and platelet-derived, epidermal, insulin-like, transforming and fibroblast growth factors. The selection of the particular cytokines will depend upon the particular modulation of the immune system that is desired. The activity of cytokines on particular cell types is known to those of ordinary skill in the art.

The precise amounts of the foregoing cytokines used in the invention will depend upon a variety of factors, including the conjugate selected, the dose amount and dose timing selected, the mode of administration, and the characteristics of the subject. The precise amounts selected can be determined without undue experimentation, particularly since a threshold amount will be any amount which will enhance the desired immune response.

Thus, it is believed that nanogram to milligram amounts of cytokines are useful, depending upon the mode of delivery, but that nanogram to microgram amounts are likely to be most useful because physiological levels of cytokines are correspondingly low.

The preparations of the invention are administered in effective amounts. An "effective amount" is that amount of a conjugate that will, alone or together with further doses, stimulate a response as desired. A "therapeutically effective amount" as used herein is that amount of a conjugate that will, alone or together with further doses, stimulate a therapeutic response as desired. In various embodiments this may involve the prevention, alleviation, or stabilization of signs or symptoms of a disease, disorder or condition of the subject.

The preferred amount of FcRn binding partner conjugates in all pharmaceutical preparations made in accordance with the present invention should be a therapeutically effective amount thereof which is also a medically acceptable amount thereof. Actual dosage levels of FcRn binding partner conjugates in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of FcRn binding partner conjugates which is effective to achieve the desired therapeutic response for a particular patient, pharmaceutical composition of FcRn binding partner conjugates, and mode of administration, without being toxic to the patient.

The selected dosage level and frequency of administration of the conjugates of the invention will depend upon a variety of factors, including the means of administration, the time of administration, the rates of excretion and metabolism of the therapeutic agent(s) including FcRn binding partner conjugates, the duration of the treatment, other drugs, compounds and/or materials used in combination with FcRn binding partner conjugates, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. For example, the dosage regimen is likely to vary with pregnant women, nursing mothers and children relative to healthy adults. The precise amounts selected can be determined without undue experimentation, particularly since a threshold amount will be any amount which will effect the desired therapeutic response. Thus, it is believed that nanogram to milligram amounts are useful, depending upon the particular therapeutic agent and the condition of the subject, but that nanogram to microgram amounts are likely to be most useful because physiological and pharmacological levels of therapeutic agents are correspondingly low.

In general it is believed that doses for central airway pulmonary administration of the conjugates of the invention will fall in the range 10 ng/kg to 500 μg/kg. For example, doses of 0.1-10 μg/kg are believed to be useful for IFN-α-Fc, and doses of 1-100 μg/kg are useful for EPO-Fc. In some instances doses of more than 25 mg may best be made in divided doses.

A physician having ordinary skill in the art can readily determine and prescribe the therapeutically effective amount of the pharmaceutical composition required. For example, the physician could start doses of FcRn binding partner conjugates employed in the pharmaceutical composition of the present invention at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

Compositions may be conveniently presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the conjugate into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the conjugate into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the conjugates of the invention, further increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer based systems such as polylactic and polyglycolic acid, polyanhydrides and polycaprolactone, wax coatings, and the like.

For administration by inhalation, the conjugate of the invention can be conveniently delivered in the form of an aerosol. As noted above, the aerosol can be generated from pressurized packs or inhalers with the use of a suitable propellant, e.g., chlorofluorocarbons, hydrochlorofluorocarbons, hydrofluorocarbons, and hydrocarbons including dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, 1,1,1,2-tetrafluoroethane, 1,1,1,2,3,3,3-heptafluoropropane, or other suitable propellant. In a preferred embodiment, the aerosol is generated by contacting a solution or suspension containing the conjugate with a vibrational element such as a piezoelectric crystal connected to a suitable energy source. Preferably the aerosol contains and delivers conjugates substantially in their native, non-denatured form. In the case of a pressurized aerosol, the

dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The invention may be further understood with reference to the following examples, which are non-limiting.

Examples

Materials. SATA, N-succinimdyl S-acetylthioacetate; sulfo-LC-SPDP, sulfosuccinimidyl 6-[3'-(2-pyridyldithio)-propionamido] hexanoate; and sulfo-SMCC, sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate were purchased from Pierce (Rockford, IL). BALB/c mice were purchased from Charles River Laboratories (Wilmington, MA).

Enzymes and Cells. All restriction and modifying enzymes were purchased from New England Biolabs (Beverly, MA) or InVitrogen (GIBCO, Gaithersburg, MD), and were used according to the manufacturers' protocols. Vent polymerase was obtained from New England Biolabs (Beverly, MA) and Expand polymerase from Roche Molecular Biochemicals (Indianapolis, IN), and both were used in their manufacturer-supplied buffers with magnesium. Shrimp alkaline phosphatase (SAP) was purchased from Roche Molecular Biochemicals (Indianapolis, IN). All oligonucleotides were synthesized and purified by Integrated DNA Technologies, Inc. (Coralville, IA). The DH5α competent cells were purchased from InVitrogen (GIBCO, Gaithersburg, MD), and were used according to the manufacturer's protocol.

Expression Vector. The mammalian expression vector pED.dC was obtained from Genetics Institute (Cambridge, MA). This vector, derived from pED4 described in Kaufman RJ et al. (1991) *Nucleic Acids Res* 19:4485-90, contains the adenovirus major late promoter, which is commonly used in expression vectors for efficient transcription, and an IgG intron for increased RNA stability and export. The vector also contains an adenovirus mRNA leader sequence, EMC virus 5' UTR (ribosome entry sequence), SV40 polyA signal, and adenovirus stability element, to increase the level of RNA and thus lead to greater expression of the target protein. The vector also contains a colE1 origin of replication for growth in bacteria, as well as the β-lactamase gene for ampicillin selection in bacteria. Finally, the vector encodes a dicistronic message. The first cistron would be the target protein, while the second cistron is

the mouse dihydrofolate reductase (dhfr) gene. The dhfr gene allows for selection and amplification of the dicistronic message in dhfr-deficient cell lines. Schimke RT (1984) *Cell* 37:705-13; Urlaub G et al (1986) *Somat Cell Mol Genet* 12:555-566.

DNA templates. The vector A_2E/X was kindly provided by H. Ploegh (Massachusetts Institute of Technology, Cambridge, MA), wt EPO-Fc was kindly provided by Wayne Lencer (Harvard Medical School, Boston, MA). Adult kidney cDNA was purchased from Clontech (Palo Alto, CA). The pGEM-T Easy vector was purchased from Promega (Madison, WI).

Oligonucleotide Primers. The following oligonucleotides (shown 5' to 3' from left to right) were used in the construction of the EPO-Fc expression vectors. The portion of each primer designed to anneal to the corresponding cDNA molecule or template is underlined.

PKF:	aaaactgcagaccaccatggtaccgtgcacg	(SEQ ID NO:18)
KXR:	cgtctagagccggcgcgggtctgagtcgg	(SEQ ID NO:19)
FCGF:	aagaattcgccggcgccgctgcggtcgacaaaactc	(SEQ ID NO:20)
FCGMR:	ttcaattgtcatttacccggagacaggg	(SEQ ID NO:21)
EPO-F:	aatctagagccccaccacgcctcatctgtgac	(SEQ ID NO:22)
EPO-R:	ttgaattctctgtcccctgtcctgcaggcc	(SEQ ID NO:23)
EPS-F:	gtacctgcaggcggagatgggggtgca	(SEQ ID NO:24)
EPS-R:	cctggtcatctgtcccctgtcc	(SEQ ID NO:25)

PCR Amplification. Polymerase chain reactions were performed in either an Idaho Technology RapidCycler or MJ Research PTC-200 Peltier Thermal Cycler.

DNA Isolation and Purification. PCR products and all restriction enzyme digestions were electrophoresed and DNA bands corresponding to the correct size were excised from an agarose gel; DNA thus excised was purified using the Qiagen DNA Purification Kit (Valencia, CA) following the manufacturer's protocol. The 1 Kb DNA ladder or 1 Kb Plus DNA ladder from Life Technologies (Rockville, MD) were used for determining the size of the DNA fragments. The concentration of the eluted DNA was estimated by visualization on an agarose gel or measurement of OD_{260}.

Ligation and Transformation. Ligation reactions were carried out using T4 DNA ligase (New England Biolabs, Beverly, MA) according to established protocols (Sambrook et. al (1989) Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor,

New York: Cold Spring Harbor Laboratory Press) or using the Rapid DNA Ligation Kit (Roche, Indianapolis, IN) according to the manufacturer's protocol. Ligation products were used for transformations of *Escherichia coli* strain DH5 according to established protocols. Sambrook et. al (1989) Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

DNA Sequencing. The sequence of the double-stranded plasmid DNA was determined by dideoxy sequencing performed at Dana Farber Molecular Biology Core Facilities (Boston, MA) or Veritas, Inc. (Rockville, MD). The sequences were compiled using SeqMan (DNAStar, Madison, WI) and additional DNA analysis was performed using the LaserGene Suite of programs (DNAStar, Madison, WI) or Vector NTI (Informax, Gaithersburg, MD).

Expression. Expression constructs were transfected into Chinese Hamster Ovary (CHO) dhfr-deficient (*dhfr-*) cell lines. Stable transfected cell lines were generated. In order to increase the EPO-Fc expression levels, the EPO-Fc gene was amplified by increasing the methotrexate concentration in the growth medium.

Example 1: Preparation of Human Immunoglobulin G

In order to prepare human IgG or human IgG fragments for the use in conjugation to a compound of the invention, e.g., an antigen or therapeutic agent, the following methods may be used. Non-specific purified human IgG may be purchased from commercial vendors such as Sigma Chemical Co., Pierce Chemical, HyClone Laboratories, ICN Biomedicals, and Organon Teknika-Cappel.

Immunoglobulin G also may be isolated by ammonium sulfate precipitation of blood serum. The protein precipitate is further fractionated by ion exchange chromatography or gel filtration chromatography to isolate substantially purified non-specific IgG. By non-specific IgG it is meant that no single antigen specificity is dominant within the antibody population or pool.

Immunoglobulin G also may be purified from blood serum by adsorption to protein A attached to a solid support such as protein A-Sepharose (Pharmacia), AvidChrom-Protein A (Sigma), or protein G-Sepharose (Sigma). Other methods of purification of IgG are well known to persons skilled in the art and may be used for the purpose of isolation of non-specific IgG.

To prepare the Fc fragments of human IgG, isolated or purified IgG are subjected to digestion with immobilized papain (Pierce) according to the manufacturer's recommended protocol. Other proteases that digest IgG to produce intact Fc fragments that can bind to Fc receptors, e.g., plasmin (Sigma) or immobilized ficin (Pierce), are known to skilled artisans and may be used to prepare Fc fragments. The digested immunoglobulin then is incubated with an affinity matrix such as protein A-Sepharose or protein G-Sepharose. Non-binding portions of IgG are eluted from the affinity matrix by extensive washing In batch or column format. Fc fragments of IgG then are eluted by addition of a buffer that is incompatible with Fc-adsorbent binding. Other methodologies effective in the purification of Fc fragments also may be employed.

Example 2: Conjugation of Compounds to Human Immunoglobulin Fc Fragments

To deliver compounds via the FcRn transport mechanism, such compounds can be coupled to whole IgG or Fc fragments. The chemistry of cross-linking and effective reagents for such purposes are well known in the art. The nature of the crosslinking reagent used to conjugate whole IgG or Fc fragments and the compound to be delivered is not restricted by the invention. Any crosslinking agent may be used provided that the activity of the compound is retained and binding by the FcRn of the Fc portion of the conjugate is not adversely affected.

An example of an effective one-step crosslinking of Fc and a compound is oxidation of Fc with sodium periodate in sodium phosphate buffer for 30 minutes at room temperature, followed by overnight incubation at 4°C with the compound to be conjugated. Conjugation also may be performed by derivatizing both the compound and Fc fragments with sulfo-LC-SPDP for 18 hours at room temperature. Conjugates also may be prepared by derivatizing Fc fragments and the desired compound to be delivered with different crosslinking reagents that will subsequently form a covalent linkage. An example of this reaction is derivatization of Fc fragments with sulfo-SMCC and the compound to be conjugated to Fc is thiolated with SATA. The derivatized components are purified free of crosslinker and combined at room temperature for one hour to allow crosslinking. Other crosslinking reagents comprising aldehyde, imide, cyano, halogen, carboxyl, activated carboxyl, anhydride and maleimide functional groups are known to persons of ordinary skill in the art and also may be used for conjugation of compounds to Fc fragments. The choice of cross-linking reagent will, of

course, depend on the nature of the compound desired to be conjugated to Fc. The crosslinking reagents described above are effective for protein-protein conjugations. If the compound to be conjugated is a carbohydrate or has a carbohydrate moiety, then heterobifunctional crosslinking reagents such as ABH, M2C2H, MPBH and PDPH are useful for conjugation with a proteinaceous FcRn-binding molecule (Pierce). Another method of conjugating proteins and carbohydrates is disclosed by Brumeanu et al. (*Genetic Engineering News*, October 1, 1995, p. 16). If the compound to be conjugated is a lipid or has a lipid moiety which is convenient as a site of conjugation for the FcRn-binding molecule, then crosslinkers such as SPDP, SMPB and derivatives thereof may be used (Pierce). It is also possible to conjugate any molecule which is to be delivered by noncovalent means. One convenient way for achieving noncovalent conjugation is to raise antibodies to the compound to be delivered, such as monoclonal antibodies, by methods well known in the art, and select a monoclonal antibody having the correct Fc region and desired antigen binding properties. The antigen or therapeutic agent to be delivered is then prebound to the monoclonal antibody carrier. In all of the above crosslinking reactions it is important to purify the derivatized compounds free of crosslinking reagent. It is important also to purify the final conjugate substantially free of unconjugated reactants. Purification may be achieved by affinity, gel filtration or ion exchange chromatography based on the properties of either component of the conjugate. A particularly preferred method is an initial affinity purification step using protein A-Sepharose to retain Fc and Fc-compound conjugates, followed by gel filtration or ion exchange chromatography based on the mass, size or charge of the Fc conjugate. The initial step of this purification scheme ensures that the conjugate will bind to FcRn which is an essential requirement of the invention.

Example 3: Construction of a General-Use X-Fc Expression Vector

The K^b signal peptide allows for efficient production and secretion of many different possible proteins fused to Fcγ1. A general-use X-Fc expression vector was therefore constructed by inserting into the first cistron position of pED.dC an expression cassette consisting of the K^b signal peptide fused to aspartic acid 221 (D221, EU numbering) in the hinge region of Fcγ1 by a 13-amino acid peptide linker (GSRPGEFAGAAAV; SEQ ID NO:26).

The K^b signal sequence was obtained from the A_2E/X template using primers PKF and

KXR in the RapidCycler using Vent polymerase, denaturing at 95°C for 15 sec, followed by 28 cycles with a slope of 6.0 of 95°C for 0 sec, 55°C for 0 sec, and 72°C for 1 min 20 sec, followed by 3 min extension at 72°C. Primer PKF contains a *Pst*I site, while primer KXR contains an *Xba*I site. The two restriction sites facilitated directional cloning of the amplified product. A PCR product of approximately 90 base pairs (bp) was gel purified, digested with *Pst*I and *Xba*I, gel purified again and subcloned into a *Pst*I/*Xba*I-digested, gel purified pED.dC vector. One construct was chosen as the representative clone and named pED.dC.K^b.

The Fcγ1 sequence was obtained from wt EPO-Fc template using primers FCGF and FCGMR in the RapidCycler using Expand polymerase, denaturing at 95°C for 15 sec, followed by 30 cycles with a slope of 6.0 of 95°C for 0 sec, 50°C for 0 sec, and 72°C for 1 min 20 sec, followed by 10 min extension at 72°C. A product of approximately 720 bp was gel-isolated and cloned into pGEM-T Easy vector and then sequenced. The correct coding region was then excised by *Eco*RI-*Mfe*I digestion, gel purified and subcloned into the *Eco*RI-digested, gel-purified pED.dC.K^b construct. The plasmid with the Fcγ coding region in the correct orientation was determined by digestion with *Sma*I, and the sequence of this construct was determined. The construct was named pED.dC.XFc. The plasmid map and partial sequence of pED.dC.XFc is shown in **Figure 3**.

Example 4: Construction of an EPO-Fc Expression Vector with K^b Signal Peptide

In this example, the mature human EPO sequence was inserted into the cassette, generating a cDNA encoding the K^b signal peptide, a 3-amino acid linker (GSR), the mature EPO sequence, and an 8-amino acid linker (EFAGAAAV, SEQ ID NO:27), followed by the Fcγ1 sequence. The EPO sequence was obtained from an adult kidney QUICK-clone cDNA preparation as the template using primers EPO-F and EPO-R in the RapidCycler using Vent polymerase, denaturing at 95°C for 15 sec, followed by 28 cycles with a slope of 6.0 of 95°C for 0 sec, 55°C for 0 sec, and 72°C for 1 min 20 sec, followed by 3 min extension at 72°C. Primer EPO-F contains an *Xba*I site, while primer EPO-R contains an *Eco*RI site. An approximately 514 bp product was gel-purified, digested with *Xba*I and *Eco*RI, gel-purified again, and directionally subcloned into an *Xba*I/*Eco*RI-digested, gel-purified pED.dC.XFc vector. Following transformation, four of the twenty clones examined possessed the correct insert. One such clone was found to be free of mutations as determined by direct sequencing. This construct was named pED.dC.EpoFc. Refer to **Figure 2** for nucleic acid and amino acid

sequences of wildtype human EPO. The plasmid map and partial sequence of pED.dC.EpoFc is shown in **Figure 4**.

Example 5: Construction of an EPO-Fc Expression Vector with EPO Signal Peptide

To evaluate the production and secretion of EPO-Fc when the endogenous EPO signal peptide was used rather than the K^b signal, a second EPO-Fc expression plasmid was generated. The secretion cassette in this plasmid encoded the human EPO sequence including its endogenous signal peptide fused to an 8-amino acid linker (EFAGAAAV, SEQ ID NO:27), followed by the Fcγ1 sequence. The native EPO sequence, containing both the endogenous signal peptide and the mature sequence, was obtained from an adult kidney QUICK-clone cDNA preparation as the template using EPS-F and EPS-R primers in the PTC-200 using Expand polymerase, denaturing at 94°C for 2 min, followed by 32 cycles of 94°C for 30 sec, 57°C for 30 sec, and 72°C for 45 sec, followed by 10 min extension at 72°C. The primer EPS-F contains an *Sbf*I site upstream of the start codon, while the primer EPS-R anneals downstream of the endogenous *SbfI* site in the EPO sequence. An approximately 603 bp product was gel-isolated and subcloned into the pGEM-T Easy vector. Four independent constructs were fully sequenced, and one of the two that were free of mutations was used for further subcloning. The correct coding sequence was excised by *Sbf*I digestion, gel-purified, and cloned into the *Pst*I-digested, SAP-treated, gel-purified pED.dC.EpoFc plasmid. The plasmid with the insert in the correct orientation was initially determined by *Kpn*I digestion. A *Xmn*I and *Pvu*II digestion of this construct was compared with pED.dC.EpoFc and confirmed the correct orientation. The sequence was determined and the construct was named pED.dC.natEpoFc. The plasmid map and partial sequence of pED.dC.natEpoFc is shown in **Figure 5**.

Example 6: Retention of Biological Activity of EPO-Fc in vivo

In order to demonstrate that a conjugate made by the fusion of an FcRn binding partner and a protein of interest is capable of retaining biological activity, the example protein above was expressed and assayed for biological activity of erythropoietin in the following manner. The mammalian expression vector containing the EPO-Fc fusion was transfected into Chinese hamster ovary (CHO) cells and expressed by standard protocols in the art. Supernatants of transfected or non-transfected CHO cells were collected and injected

subcutaneously into BALB/c mice. Reticulocyte counts of mice were obtained by Coulter FACS analysis by techniques known in the field of the art. Results demonstrated that mice injected with the supernatants of the transfected cells had reticulocyte counts several fold higher than mice injected with control (untransfected) supernatants. Since EPO has been documented to stimulate the production of erythrocytes, the results disclosed herein support the ability of the invention to synthesize biologically active FcRn binding partner conjugates.

Similarly, fusion proteins substituting the Fc fragment for an alternate FcRn binding partner domain in the vector described above would be expected to retain biological activity.

Example 7: Transepithelial Absorption of EPO-Fc after Delivery to Central Airways

Immunohistochemical studies showed that FcRn is expressed at relatively higher levels in the central airways than in the alveolar epithelium in both cynomolgus monkeys and humans. Therefore, it was of interest to determine whether an EPO-Fc fusion protein (MW = 112 kDa) that binds to FcRn can be transported through the lung epithelium and where in the lung this absorption occurs. A human EPO-Fc fusion protein, comprised of native human EPO fused at its carboxyl terminus to the amino terminus of the Fc domain of human IgG1, was expressed in CHO cells and purified from the cell culture medium using Protein A affinity chromatography. The purified human EPO-Fc fusion protein was biologically active in vitro. EPO-Fc bound to the EPO receptor (EpoR) with high affinity (K_d = 0.25 nM vs. 0.2 nM for native huEPO) and stimulated the proliferation of TF-1 human erythroleukemia cells (ED_{50} = 0.07 nM vs. 0.03 nM for native huEPO). EPO-Fc also bound to purified, soluble huFcRn (K_d = 14 nM vs. 8 nM for IgG1) in a Biacore assay.

Aerosols of EPO-Fc (in PBS, pH 7.4) were created with various jet nebulizers and administered to anesthetized cynomolgus monkeys through endotracheal tubes. In some experiments monkeys were breathing spontaneously, while in other experiments the depth and rate of respiration were regulated with either a Bird Mark 7A respirator or a Spangler box apparatus. An increase in circulating reticulocytes was used as an indicator of the biological response to EPO-Fc. EPO-Fc was quantified in serum using a specific ELISA.

Initial studies in anesthetized, spontaneously breathing cynomolgus monkeys examined the biological response to aerosolized EPO-Fc (**Figure 6A**). All animals in this study responded with an increase in circulating reticulocytes, 5-7 days after EPO-Fc administration. Subsequent studies showed that high concentrations of EPO-Fc were

obtained in serum after single doses administered in a similar manner (**Figure 6B**). A mutated EPO-Fc (Fc modified in three critical amino acid residues in the Fc domain: I253A, H310A, and H435A) that is reduced in its FcRn binding by >90%, was not well absorbed. Mean serum half-life was approximately 22 hr for EPO-Fc (compared to 5-6 hr for EPOGEN® (Amgen)). The absorption of EPO-Fc and the mutEPO-Fc was compared using either shallow (spontaneous) breathing or deep (forced ventilation) breathing. Forced, deep breathing maneuvers resulted in much less absorption of EPO-Fc than shallow, spontaneous breathing, while there was no difference in absorption of mutated EPO-Fc.

These results were confirmed and enhanced in an experiment using gamma scintigraphy (co-administration of ^{99m}Tc-DTPA as a radiotracer) to compare deposition and absorption of EPO-Fc with forced ventilation at either 20% or 75% vital capacity (**Figure 7**). Scintigraphic images demonstrated that deposition of radiotracer was tracheal/central airway for 20% vital capacity vs. central airway/deep lung for 75% vital capacity. Absorption of EPO-Fc was more robust after administration using 20% of vital capacity. Additionally, the absorption of EPO-Fc was examined at different deposited dose levels (all done with 20% vital capacity maneuvers) to find a dose range for EPO-Fc that is clinically relevant. Deposited doses of 0.01-0.03 mg/kg resulted in pharmacokinetics consistent with clinical utility (**Figure 8**).

Example 8. Systemic Delivery of IFN-α by Aerosol Administration of Human IFN-α-Fc to Central Airways of Non-Human Primates

A human IFN-α-Fc expression construct was created using the pED.dC.K^{b} expression vector of Example 3 and the coding region of human IFN-α. The nucleotide sequence for human IFN-α is publicly available from GenBank as accession no. J00207. Human IFN-α-Fc was expressed in CHO cells and isolated in a manner analogous to that for EPO-Fc as described above. Six cynomolgus monkeys were divided into three groups for this experiment. Group I monkeys were administered 20 μg/kg of IFN-α-Fc by central airways aerosol administration analogous to the methods described for EPO-Fc administration in Example 7. Group II monkeys were administered 20 μg/kg of INTRON® A (Schering Corporation, Kenilworth, NJ), recombinant human IFN-α, to central airways in the same manner. Group III monkeys were administered one tenth as much IFN-α-Fc as Group I, i.e.,

2 μg/kg, by central airways aerosol administration. Blood samples were drawn periodically over 14 days and serum levels of IFN-α were determined at each time point using an appropriate specific ELISA. Pretreatment IFN-α levels, also determined by the same ELISA, were subtracted from all subsequent IFN-α level determinations. In addition, standard assays for bioactivity of IFN-α were performed using serial samples obtained from the animals in group I in order to assess bioactivity of the administered IFN-α-Fc. These assays included measurements of oligoadenylate synthetase (OAS) activity and of neopterin concentration. Results are shown in **Figures 9-11**.

Figure 9 shows that monkeys in Group I (DD030 and DD039) achieved peak serum concentrations of IFN-α in the range of 160-185 ng/ml, with a half-life ($T_{½}$) of 83.7-109 hours. In contrast, monkeys in Group II (DD029 and DD045), receiving 20 μg/kg of IFN-α as INTRON® A in the same manner of administration, achieved peak serum levels of IFN-α of only about 13.6 ng/ml, with a half-life ($T_{½}$) of only 4.8-5.9 hours. These results indicate that aerosolized IFN-α-Fc administered to central airways is highly effective for systemic delivery of IFN-α. In addition, the prolonged half-life of IFN-α, thus adminsitered as IFN-α-Fc, demonstrates that IFN-α can be administered as an FcRn binding partner conjugate with dramatically improved pharmacokinetics compared to similarly administered IFN-α alone.

Figure 10 shows that monkeys in Group III (DD055 and DD057), administered only on tenth as much IFN-α-Fc as monkeys in Group I, achieved proportionately lower serum concentrations with a similar pharmacokinetics profile.

Figure 11 shows the results of IFN-α bioactivity assays for Group I monkeys receiving IFN-α-Fc. **Figure 11A** shows the increased and sustained OAS activity as a function of time paralleled the pharmacokinetic data in **Figure 9** and **Figure 10**. **Figure 11B** shows the increased and sustained neopterin concentration also paralleled the pharmacokinetic data in in **Figure 9** and **Figure 10**. These data indicate that IFN-α in the IFN-α-Fc retains biological activity following aerosol administration to central airways according to the methods of the invention.

Example 9. Systemic Delivery of TNFR-Fc by Aerosol Administration of Human TNFR-Fc to Central Airways of Non-Human Primates

Each of three cynomolgus monkeys was administered aerosolized ENBREL® (etanercept, Immunex Corporation, Seattle, WA), recombinant human tumor necrosis factor receptor (TNFR)-Fcγ1, via the central airways according to the methods of the instant invention. ENBREL® is a dimeric fusion protein that includes the extracellular ligand-binding portion of human TNFR fused in frame to the hinge, C_H2, C_H3 domains of human IgG1. ENBREL® is expressed in CHO cells and has an approximate molecular weight of 150 kDa. The estimated deposited dose for each monkey in this experiment was 0.3-0.5 mg/kg. Blood samples were drawn periodically over ten days and serum levels of TNFR-Fc were determined at each time point using an appropriate specific ELISA. For the measurement of serum ENBREL® concentrations, a sandwich ELISA was performed using TNF-α bound to the plate as capture agent; serum or ENBREL® as the sample or standard, respectively; and anti-TNFR antibody as reporter agent. Results are shown in **Figure 12.**

Figure 12 shows that the three cynomolgus monkeys (101, 102, and 103) achieved similar peak serum concentrations of TNFR-Fc of about 200 ng/ml. The half-life of the TNFR-Fc was prolonged. This experiment demonstrates that human TNFR-Fc can be effectively administered to non-human primates via aerosol admininstration to the central airways according to the methods of the instant invention.

Example 10. Systemic Delivery of IFN-β by Aerosol Administration of Human IFN-β-Fc to Central Airways of Non-Human Primates

A human IFN-β-Fc expression construct was created using the pED.dC.K^b expression vector of Example 3 and the coding region of human IFN-β. The nucleotide sequence for human IFN-β is publicly available from GenBank as accession no. V00535. Human IFN-β-Fc was expressed in CHO cells and isolated in a manner analogous to that for EPO-Fc as described above. Two cynomolgus monkeys and two rhesus monkeys each were administered 40 μg/kg of IFN-β-Fc by central airway aerosol administration analogous to the methods described for EPO-Fc administration in Example 7. Blood samples were drawn periodically over two days and serum levels of IFN-β were determined at each time point using an appropriate specific ELISA. Pretreatment IFN-β levels, also determined by the same ELISA, were subtracted from all subsequent IFN-β level determinations.

Results showed that both cynomolgus and rhesus monkeys administered aerosolized

human IFN-β-Fc via the central airways achieved significant and sustained serum concentrations of IFN-β. The cynomolgus monkeys in this experiment achieved higher peak levels than did the rhesus monkeys (11.0-24.7 ng/ml for cynomolgus versus 5.4-8.4 ng/ml for rhesus). The half-life of IFN-β-Fc in both groups was about the same, i.e., 12.8-14.2 hours. These data demonstrate that aerosolized IFN-β-Fc administered to central airways of two species of non-human primates is effective for systemic delivery of IFN-β.

Example 11. Systemic Delivery of FSH by Aerosol Administration of Human FSH-Fc to Central Airways of Non-Human Primates

A human FSH-Fc expression construct was created using the pED.dC.K^b expression vector of Example 3 and the coding region of a single-chain human FSH. The single chain FSH portion of the molecule includes both the α and the β chains of the heterodimeric hormone FSH, linked together in proper translational reading frame by a *Sma* I restriction endonuclease site (CCCGGG). The FSH-Fc construct is thus also referred to as hFSHβα-Fc. The nucleotide sequences for α and β subunits of human FSH are publicly available through GenBank as accession numbers NM_000735 and NM_000510, respectively. Human FSH-Fc was expressed in CHO cells and isolated in a manner analogous to that for EPO-Fc as described above.

Two cynomolgus monkeys were each administered 100 μg/kg of FSH-Fc by central airway aerosol administration analogous to the methods described for EPO-Fc administration in Example 7. Blood samples were drawn periodically over two weeks and serum levels of FSH were determined at each time point using appropriate specific ELISA. Pretreatment FSH levels, also determined by the same ELISA, were subtracted from all subsequent FSH level determinations. Results showed that both monkeys achieved significant levels of FSH, with peak serum concentrations of 21.6 and 42.8 ng/ml with a half-life of 145-153 hours.

The invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed various modifications of the invention, in addition to those shown and described herein, will become apparent to those skilled in the art from the foregoing description and

accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All references cited herein are incorporated herein in their entirety by reference for all purposes.

We claim:

Claims

1. A method for systemic delivery of a therapeutic agent, comprising:

administering an effective amount of an aerosol of a conjugate of a therapeutic agent and an FcRn binding partner to lung such that a central lung zone/peripheral lung zone deposition ratio (C/P ratio) is at least 0.7.

2. The method of claim 1, wherein the C/P ratio is at least 1.0.

3. The method of claim 1, wherein the C/P ratio is at least 1.5.

4. The method of claim 1, wherein the C/P ratio is at least 2.0.

5. The method of claim 1, wherein the therapeutic agent is a polypeptide.

6. The method of claim 1, wherein the therapeutic agent is an antigen.

7. The method of claim 6, wherein the antigen is a tumor antigen.

8. The method of claim 1, wherein the therapeutic agent is an oligonucleotide.

9. The method of claim 8, wherein the oligonucleotide is an antisense oligonucleotide.

10. The method of claim 1, wherein the therapeutic agent is erythropoietin (EPO), growth hormone, interferon alpha (IFN-α), interferon beta (IFN-β), or follicle stimulating hormone (FSH).

11. The method of claim 1, wherein the therapeutic agent is EPO.

12. A method for systemic delivery of a therapeutic agent, comprising:

administering an effective amount of an aerosol of a conjugate of a therapeutic agent and an FcRn binding partner to lung, wherein particles in the aerosol have a mass median aerodynamic diameter (MMAD) of at least 3 micrometers (μm).

13. The method of claim 12, wherein the MMAD of the particles is between 3 μm and about 8 μm.

14. The method of claim 12, wherein the MMAD of the particles is greater than 4 μm.

15. The method of claim 12, wherein a majority of the particles are non-respirable.

16. The method of claim 12, wherein the therapeutic agent is a polypeptide.

17. The method of claim 12, wherein the therapeutic agent is an antigen.

18. The method of claim 17, wherein the antigen is a tumor antigen.

19. The method of claim 12, wherein the therapeutic agent is an oligonucleotide.

20. The method of claim 19, wherein the oligonucleotide is an antisense oligonucleotide.

21. The method of claim 12, wherein the therapeutic agent is EPO, growth hormone, IFN-α, IFN-β, or FSH.

22. The method of claim 12, wherein the therapeutic agent is EPO.

23. An aerosol of a conjugate of a therapeutic agent and an FcRn binding partner, wherein particles in the aerosol have a MMAD of at least 3 μm.

24. The aerosol of claim 23, wherein the MMAD of the particles is between 3 μm and about 8 μm.

25. The aerosol of claim 23, wherein the MMAD of the particles is greater than 4 μm.

26. The aerosol of claim 23, wherein a majority of the particles are non-respirable.

27. The aerosol of claim 23, wherein the therapeutic agent is a polypeptide.

28. The aerosol of claim 23, wherein the therapeutic agent is an antigen.

29. The aerosol of claim 28, wherein the antigen is a tumor antigen.

30. The aerosol of claim 23, wherein the therapeutic agent is an oligonucleotide.

31. The aerosol of claim 30, wherein the oligonucleotide is an antisense oligonucleotide.

32. The aerosol of claim 23, wherein the therapeutic agent is EPO, growth hormone, IFN-α, IFN-β, or FSH.

33. The aerosol of claim 23, wherein the therapeutic agent is EPO.

34. An aerosol delivery system, comprising a container, an aerosol generator connected to the container, and a conjugate of a therapeutic agent and an FcRn binding partner disposed within the container, wherein the aerosol generator is constructed and arranged to generate an aerosol of the conjugate having particles with a MMAD of at least 3 μm.

35. The aerosol delivery system of claim 34, wherein the MMAD of the particles is greater than 4 μm.

36. The aerosol delivery system of claim 34, wherein a majority of the particles are non-respirable.

37. The aerosol delivery system of claim 34, wherein the aerosol generator comprises a vibrational element in fluid connection with a solution containing the conjugate.

38. The aerosol delivery system of claim 34, wherein the aerosol generator is a nebulizer.

39. The aerosol delivery system of claim 34, wherein the aerosol generator is a mechanical pump.

40. The aerosol delivery system of claim 34, wherein the container is a pressurized container.

41. A method of manufacturing the aerosol delivery system of claim 34, comprising:
providing the container;
providing the aerosol generator connected to the container; and
placing an effective amount of the conjugate in the container.

42. The method of claim 41, wherein the the aerosol generator comprises a vibrational element in fluid connection with a solution containing the conjugate.

43. The method of claim 41, wherein the aerosol generator is a nebulizer

44. The method of claim 41, wherein the aerosol generator is a mechanical pump.

45. The method of claim 41, wherein the container is a pressurized container.

Figure 1

```
gacaaaactcacacatgtccaccttgtccagctccggaactcctggggggaccgtcagtc    60
 D  K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V     20
221            226            231            236

ttcctcttccccccaaaacccaaggacaccctcatgatctcccggacccctgaggtcaca   120
 F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T     40
241            246            251            256

tgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggac   180
 C  V  V  V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D     60
261            266            271            276

ggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtac   240
 G  V  E  V  H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y     80
281            286            291            296

cgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaag   300
 R  V  V  S  V  L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K    100
301            306            311            316

tgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaa   360
 C  K  V  S  N  K  A  L  P  A  P  I  E  K  T  I  S  K  A  K    120
321            326            331            336

gggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaag   420
 G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  D  E  L  T  K    140
341            346            351            356

aaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggag   480
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361            366            371            376

tgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactcc   540
 W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S    180
381            386            391            396

gacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcagggg   600
 D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G    200
401            406            411            416

aacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc   660
 N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S    220
421            426            431            436

ctctccctgtctccgggtaaa                                          681
 L  S  L  S  P  G  K                                           227
441            446
```

Figure 2

A SEQ ID NO:3

```
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aggtacctct tggaggccaa ggaggccgag aatatcacga cgggctgtgc tgaacactgc   180
agcttgaatg agaatatcac tgtcccagac accaaagtta atttctatgc ctggaagagg   240
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gtcctgcggg gccaggccct gttggtcaac tcttcccagc cgtgggagcc cctgcagctg   360
catgtggata aagccgtcag tggccttcgc agcctcacca ctctgcttcg ggctctggga   420
gcccagaagg aagccatctc ccctccagat gcggcctcag ctgctccact ccgaacaatc   480
actgctgaca ctttccgcaa actcttccga gtctactcca atttcctccg gggaaagctg   540
aagctgtaca caggggaggc ctgcaggaca ggggacaga                          579
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B SEQ ID NO:4

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SLNENITVPD TKVNFYAWKR MEVGQQAVEV WQGLALLSEA VLRGQALLVN SSQPWEPLQL   120
HVDKAVSGLR SLTTLLRALG AQKEAISPPD AASAAPLRTI TADTFRKLFR VYSNFLRGKL   180
KLYTGEACRT GDR                                                      193
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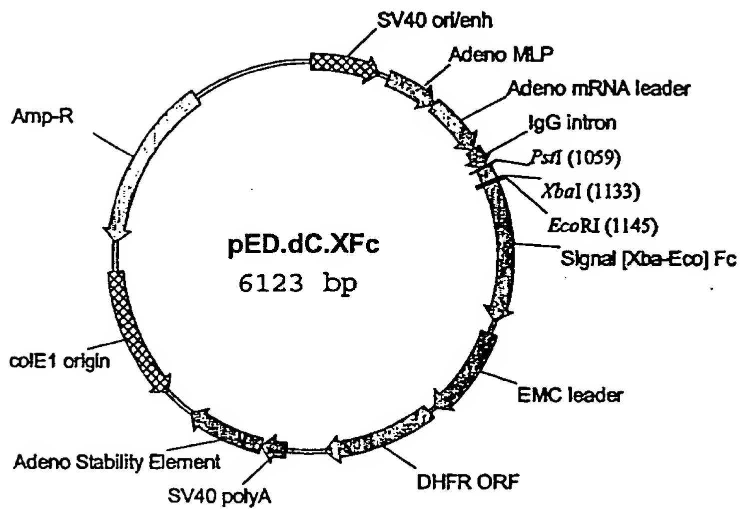
Figure 3

A



B

K^b Signal peptide

1 M V P C T
1054 ctgcagaccaccatggtaccgtgcacg
PstI

K^b Signal peptide

6 L L L L L A A A L A P T Q T R A G S R P
1081 ctgctcctgctgttggcggccgccctggctccgactcagacccgcgccggctctagaccc
XbaI

Fcγ1

26 G E F A G A A A V D K T H T C P P C P A
1141 ggggaattcgccggcgccgctgcggtcgacaaaactcacacatgcccaccgtgcccagca
EcoRI

Fcγ1

46 P E L L G G P S V F L F P P K P K D T L
1201 cctgaactcctggggggaccgtcagtcttcctcttccccccaaaacccaaggacaccctc

Fcγ1

66 M I S R T P E V T C V V V D V S H E D P
1261 atgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccct

Fcγ1

86 E V K F N W Y V D G V E V H N A K T K P
1321 gaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccg

```
                               Fcγ1
     ------------------------------------------------------------
 106 R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q
1381 cgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccag

                               Fcγ1
     ------------------------------------------------------------
 126 D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A  P
1441 gactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccc

                               Fcγ1
     ------------------------------------------------------------
 146 I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V  Y  T  L
1501 atcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg

                               Fcγ1
     ------------------------------------------------------------
 166 P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L  V  K  G
1561 cccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggc

                               Fcγ1
     ------------------------------------------------------------
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1621 ttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactac

                               Fcγ1
     ------------------------------------------------------------
 206 K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T
1681 aagaccacgcctcccgtgttggactccgacggctccttcttcctctacagcaagctcacc

                               Fcγ1
     ------------------------------------------------------------
 226 V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M  H  E  A
1741 gtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggct

                         Fcγ1
     ------------------------------------------------
 246 L  H  N  H  Y  T  Q  K  S  L  S  L  S  P  G  K                 (SEQ ID NO:6)
1801 ctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga             (SEQ ID NO:5)
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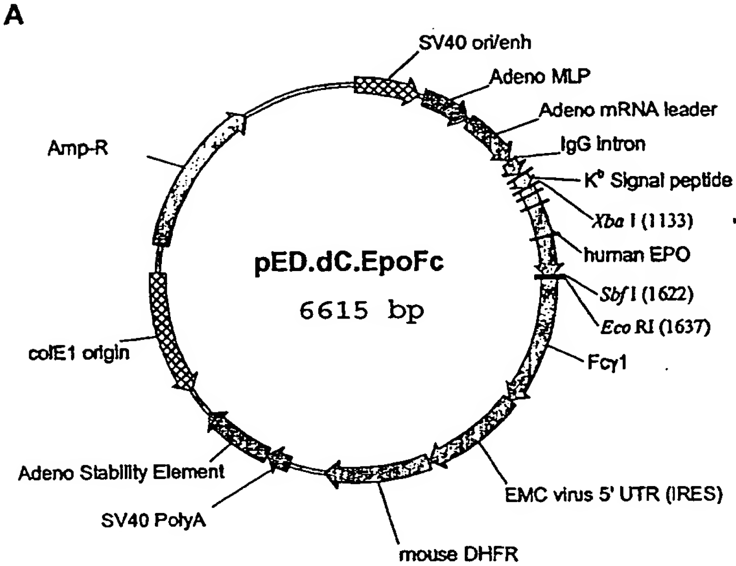
Figure 4

A



B

```
                                        Kb Signal peptide
                                        ----------------
   1                                      M  V  P  C  T
1054                               ctgcagaccaccatggtaccgtgcacg
                                   PstI

                    Kb Signal peptide                        EPO
     ---------------------------------------------------      ---
   6 L  L  L  L  L  A  A  A  L  A  P  T  Q  T  R  A  G  S  R  A
1081 ctgctcctgctgttggcggccgccctggctccgactcagacccgcgccggctctagagcc
                                                          XbaI

                               EPO
     ------------------------------------------------------------
  26 P  P  R  L  I  C  D  S  R  V  L  Q  R  Y  L  L  E  A  K  E
1141 ccaccacgcctcatctgtgacagccgagtcctgcagaggtacctcttggaggccaaggag

                               EPO
     ------------------------------------------------------------
  46 A  E  N  I  T  T  G  C  A  E  H  C  S  L  N  E  N  I  T  V
1201 gccgagaatatcacgacgggctgtgctgaacactgcagcttgaatgagaatatcactgtc

                               EPO
     ------------------------------------------------------------
  66 P  D  T  K  V  N  F  Y  A  W  K  R  M  E  V  G  Q  Q  A  V
1261 ccagacaccaaagttaatttctatgcctggaagaggatggaggtcgggcagcaggccgta

                               EPO
     ------------------------------------------------------------
  86 E  V  W  Q  G  L  A  L  L  S  E  A  V  L  R  G  Q  A  L  L
1321 gaagtctggcagggcctggccctgctgtcggaagctgtcctgcggggccaggccctgttg
```

```
                                    EPO
     ---------------------------------------------------------------
 106 V   N   S   S   Q   P   W   E   P   L   Q   L   H   V   D   K   A   V   S   G
1381 gtcaactcttcccagccgtgggagcccctgcagctgcatgtggataaagccgtcagtggc

                                    EPO
     ---------------------------------------------------------------
 126 L   R   S   L   T   T   L   L   R   A   L   G   A   Q   K   E   A   I   S   P
1441 cttcgcagcctcaccactctgcttcgggctctgggagcccagaaggaagccatctcccct

                                    EPO
     ---------------------------------------------------------------
 146 P   D   A   A   S   A   A   P   L   R   T   I   T   A   D   T   F   R   K   L
1501 ccagatgcggcctcagctgctccactccgaacaatcactgctgacactttccgcaaactc

                                    EPO
     ---------------------------------------------------------------
 166 F   R   V   Y   S   N   F   L   R   G   K   L   K   L   Y   T   G   E   A   C
1561 ttccgagtctactccaatttcctccggggaaagctgaagctgtacacaggggaggcctgc
                                                              SbfI

          EPO                                            Fcγ1
     ---------------                                ---------------------
 186 R   T   G   D   R   E   F   A   G   A   A   A   V   D   K   T   H   T   C   P
1621 aggacaggggacagagaattcgccggcgccgctgcggtcgacaaaactcacacatgccca
                     EcoRI

                                    Fcγ1
     ---------------------------------------------------------------
 206 P   C   P   A   P   E   L   L   G   G   P   S   V   F   L   F   P   P   K   P
1681 ccgtgcccagcacctgaactcctggggggaccgtcagtcttcctcttccccccaaaaccc

                                    Fcγ1
     ---------------------------------------------------------------
 226 K   D   T   L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V   S
1741 aaggacaccctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagc

                                    Fcγ1
     ---------------------------------------------------------------
 246 H   E   D   P   E   V   K   F   N   W   Y   V   D   G   V   E   V   H   N   A
1801 cacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcc

                                    Fcγ1
     ---------------------------------------------------------------
 266 K   T   K   P   R   E   E   Q   Y   N   S   T   Y   R   V   V   S   V   L   T
1861 aagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcacc

                                    Fcγ1
     ---------------------------------------------------------------
 286 V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S   N   K   A
1921 gtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagcc

                                    Fcγ1
     ---------------------------------------------------------------
 306 L   P   A   P   I   E   K   T   I   S   K   A   K   G   Q   P   R   E   P   Q
1981 ctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacag

                                    Fcγ1
     ---------------------------------------------------------------
 326 V   Y   T   L   P   P   S   R   D   E   L   T   K   N   Q   V   S   L   T   C
2041 gtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgc

                                    Fcγ1
     ---------------------------------------------------------------
 346 L   V   K   G   F   Y   P   S   D   I   A   V   E   W   E   S   N   G   Q   P
2101 ctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccg
```

```
                                Fcγ1
     ------------------------------------------------------------
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                                Fcγ1
     ------------------------------------------------------------
 386 S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V
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                                Fcγ1
     ------------------------------------------------------------
 406 M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P  G  K    (SEQ ID NO:8)
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2341 tga                                                             (SEQ ID NO:7)
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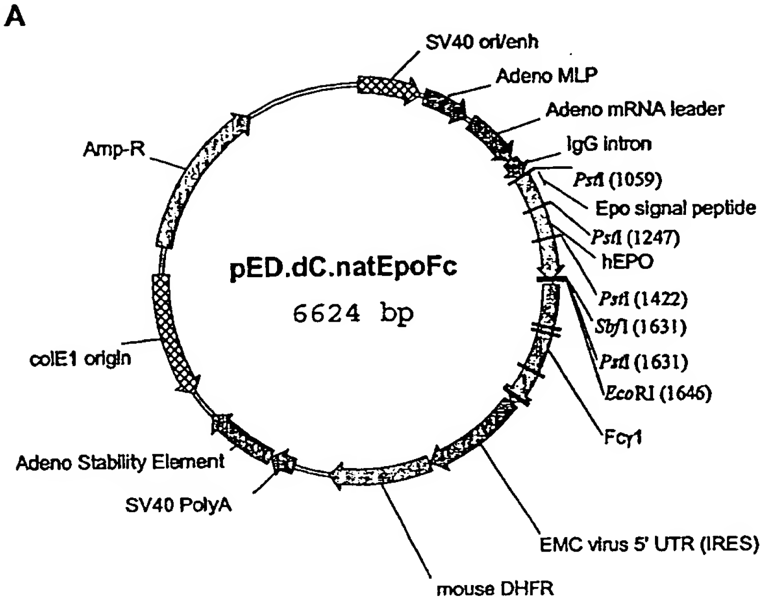
Figure 5

A



B

```
                                                      EPO
                                                -------------
   1                                            M  G  V  H  E
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                                           PstI
                          EPO
     -----------------------------------------------------------
   6 C  P  A  W  L  W  L  L  L  S  L  L  S  L  P  L  G  L  P  V
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                          EPO
     -----------------------------------------------------------
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                          EPO
     -----------------------------------------------------------
  46 A  K  E  A  E  N  I  T  T  G  C  A  E  H  C  S  L  N  E  N
1201 gccaaggaggccgagaatatcacgacgggctgtgctgaacactgcagcttgaatgagaat
                                                 PstI
                          EPO
     -----------------------------------------------------------
  66 I  T  V  P  D  T  K  V  N  F  Y  A  W  K  R  M  E  V  G  Q
1261 atcactgtcccagacaccaaagttaatttctatgcctggaagaggatggaggtcgggcag
                          EPO
     -----------------------------------------------------------
  86 Q  A  V  E  V  W  Q  G  L  A  L  L  S  E  A  V  L  R  G  Q
1321 caggccgtagaagtctggcagggcctggccctgctgtcggaagctgtcctgcggggccag
```

```
                                EPO
     ------------------------------------------------------------
 106 A  L  L  V  N  S  S  Q  P  W  E  P  L  Q  L  H  V  D  K  A
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                                          PstI

                                EPO
     ------------------------------------------------------------
 126 V  S  G  L  R  S  L  T  T  L  L  R  A  L  G  A  Q  K  E  A
1441 gtcagtggccttcgcagcctcaccactctgcttcgggctctgggagcccagaaggaagcc

                                EPO
     ------------------------------------------------------------
 146 I  S  P  P  D  A  A  S  A  A  P  L  R  T  I  T  A  D  T  F
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                                EPO
     ------------------------------------------------------------
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              EPO                                       Fcγ1
     ------------------------                      ------------
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         SbfI/PstI            EcoRI

                                Fcγ1
     ------------------------------------------------------------
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                                Fcγ1
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                                Fcγ1
     ------------------------------------------------------------
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                                Fcγ1
     ------------------------------------------------------------
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                                Fcγ1
     ------------------------------------------------------------
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```

```
                        Fcγ1
     -----------------------------------------------------------
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                        Fcγ1
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                        Fcγ1
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                        Fcγ1
     -----------------------------------------------------------
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2341 ccgggtaaatga                                     (SEQ ID NO:9)
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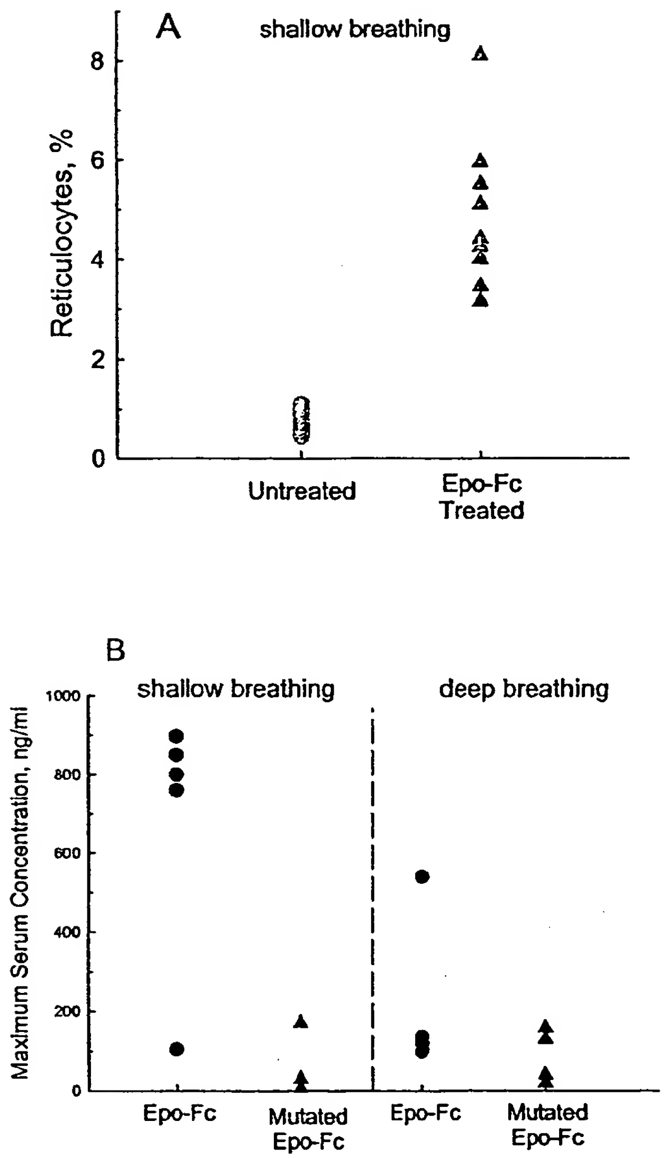
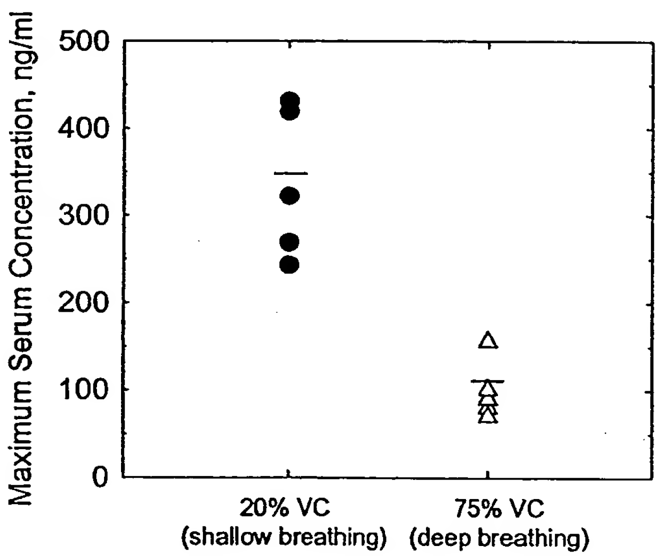
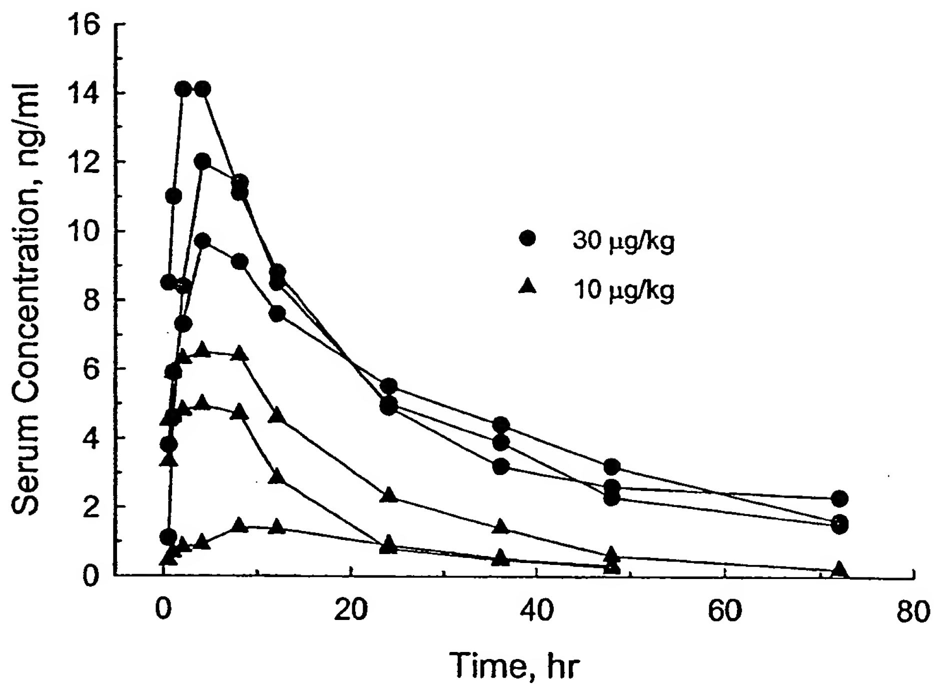

A
shallow breathing
Reticulocytes, %
8
6
4
2
0
Untreated
Epo-Fc
Treated
B
shallow breathing
deep breathing
Maximum Serum Concentration, ng/ml
1000
800
600
400
200
0
Epo-Fc
Mutated
Epo-Fc
Epo-Fc
Mutated
Epo-Fc

Figure 6



Maximum Serum Concentration, ng/ml
500
400
300
200
100
0
20% VC
(shallow breathing)
75% VC
(deep breathing)

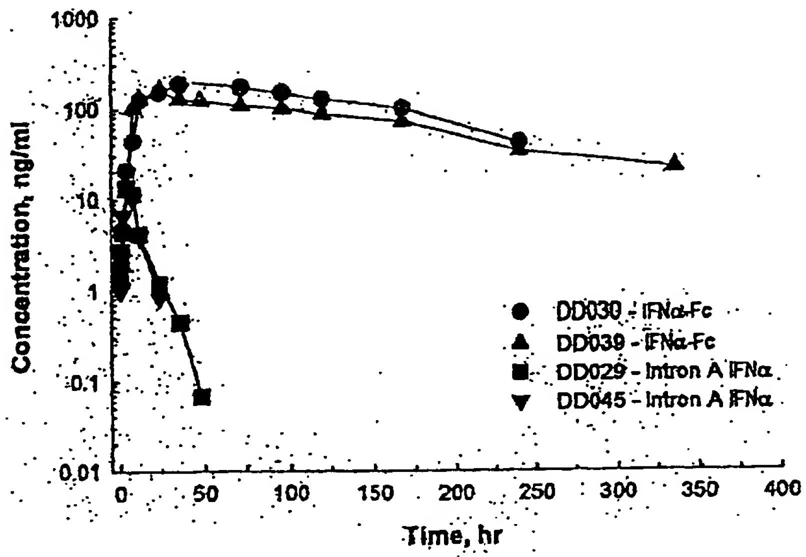
Figure 7



Serum Concentration, ng/ml
16
14
12
10
8
6
4
2
0
0
20
40
60
80
Time, hr
30 μg/kg
10 μg/kg

Figure 8

Figure 9



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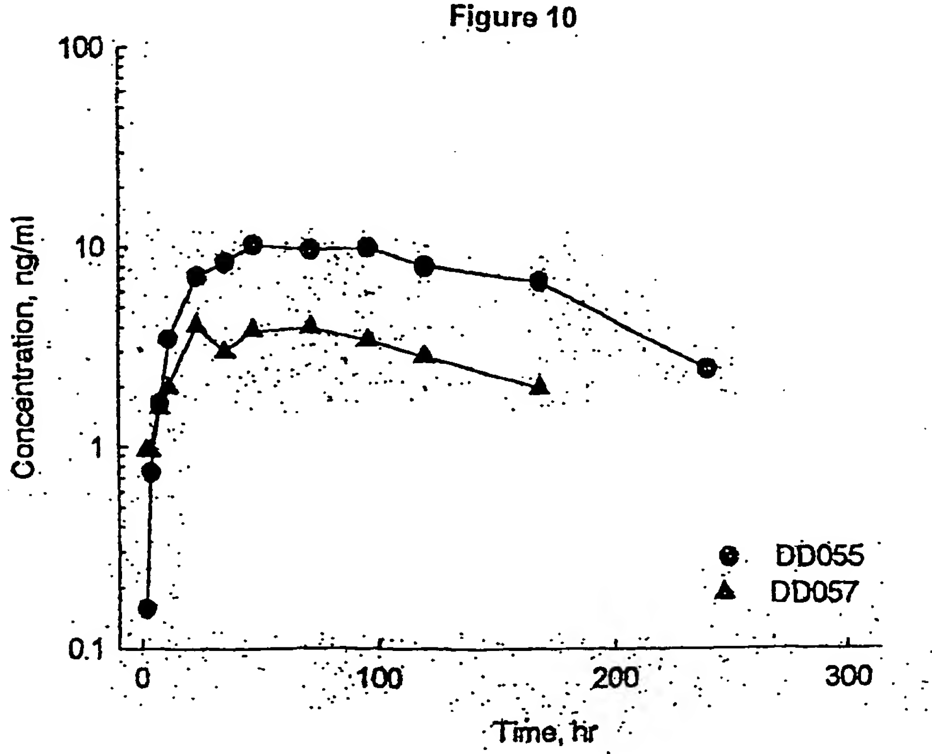


Figure 11

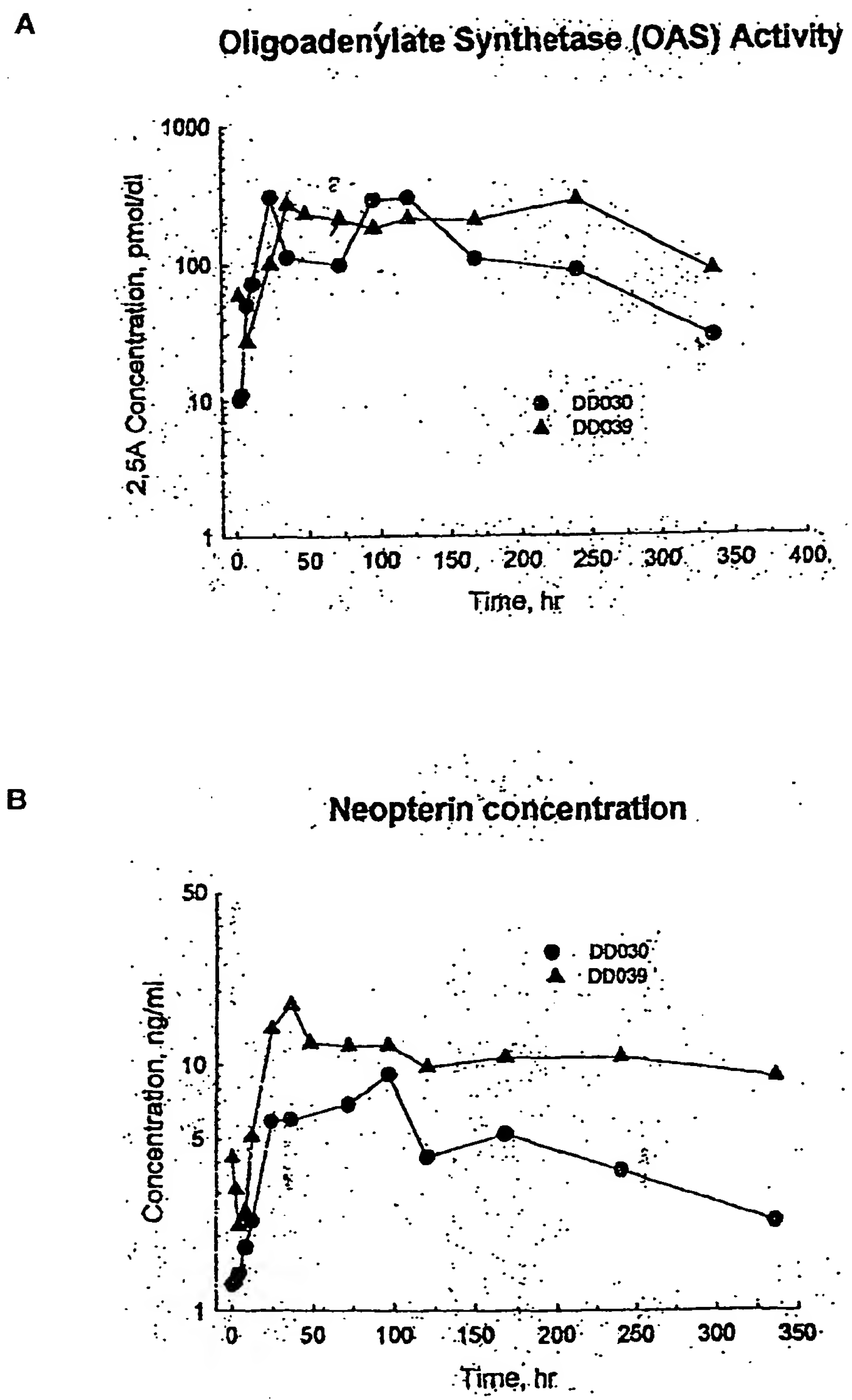
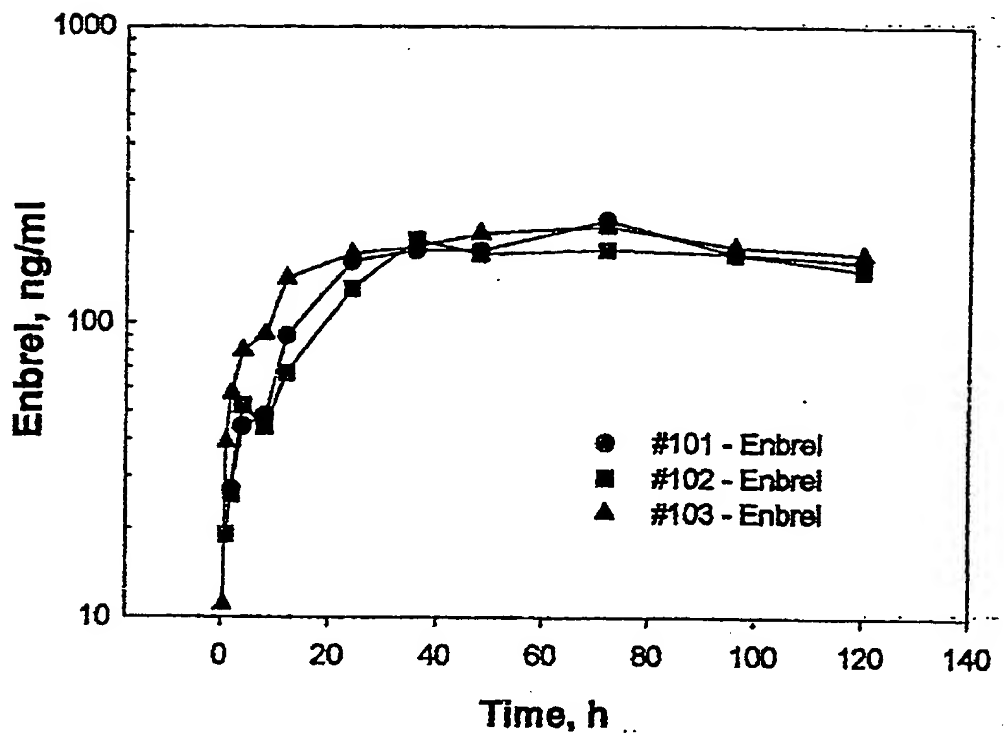

A
Oligoadenylate Synthetase (OAS) Activity
2,5A Concentration, pmol/dl
1000
100
10
1
0
50
100
150
200
250
300
350
400
Time, hr
DD030
DD039
B
Neopterin concentration
Concentration, ng/ml
50
10
5
1
0
50
100
150
200
250
300
350
Time, hr
DD030
DD039

Figure 12


1000
100
10
Enbrel, ng/ml
0
20
40
60
80
100
120
140
Time, h
#101 - Enbrel
#102 - Enbrel
#103 - Enbrel

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<120> CENTRAL AIRWAY ADMINISTRATION FOR SYSTEMIC DELIVERY OF THERAPEUTICS

<130> S01383/70005WO

<150> US 60/364,482
<151> 2002-03-15

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cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 300

tgcaaggtct ccaacaaagc cctcccagcc cccatcgaga aaaccatctc caaagccaaa 360

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35 40 45

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
50 55 60

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65 70 75 80

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
85 90 95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
100 105 110

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
115 120 125

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
130 135 140

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145 150 155 160

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
165 170 175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
180 185 190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
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<213>  Homo sapiens

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            20                  25                  30


Ile Cys Asp Ser Arg Val Leu Gln Arg Tyr Leu Leu Glu Ala Lys Glu
        35                  40                  45


Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu
    50                  55                  60


Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg
65                  70                  75                  80


Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu
                85                  90                  95


Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser
            100                 105                 110


Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly
        115                 120                 125
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20 25 30

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35 40 45

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
50 55 60

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
65 70 75 80

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
85 90 95

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
100 105 110

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
115 120 125

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
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Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
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Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
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195 200 205

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210 215 220

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Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu
35 40 45

Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu
50 55 60

Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg
65 70 75 80

Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu
85 90 95

Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser
100 105 110

Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly
115 120 125

Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu
130 135 140

Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile
145 150 155 160

Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu
165 170 175

Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp
180 185 190

Arg Glu Phe Ala Gly Ala Ala Ala Val Asp Lys Thr His Thr Cys Pro
195 200 205

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
210 215 220

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val

225 230 235 240

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
245 250 255

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
260 265 270

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
275 280 285

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
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Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
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Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
325 330 335

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
340 345 350

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
355 360 365

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
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Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
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